# STUDY ON SEROPREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION IN PATIENTS WITH CHRONIC LIVER DISEASE ATTENDING A TERTIARY CARE HOSPITAL

**Dissertation submitted in**

**Partial fulfillment of the Regulations required for the award of**

**M.D. DEGREE**

**In**

**MICROBIOLOGY– BRANCH IV**

**The Tamil Nadu**

**DR. M.G.R. MEDICAL UNIVERSITY**

**Chennai**

**APRIL 2017.**

# CERTIFICATE

This is to certify that the enclosed work **"Study on Seroprevalence and Genotypes of Hepatitis C Virus Infection in Patients with Chronic Liver Disease Attending a Tertiary Care Hospital"** submitted by **Dr.V.M.Theeba** to The Tamilnadu Dr. MGR Medical University is based on bonafide cases studied and analysed by the candidate in the Department of Microbiology, Coimbatore Medical College Hospital during the period from August 2015 to July 2016 under the guidance and supervision of **Dr. A. Dhanasekaran DCH., MD.,** Professor & HOD, Department of Microbiology and the conclusion reached in this study are her own.

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# DECLARATION

I, **Dr. V. M. Theeba,** solemnly declare that the dissertation entitled **"STUDY ON SEROPREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION IN PATIENTS WITH CHRONIC LIVER CISEASE ATTENDING A TERTIARY CARE HOSPITAL**" was done by me at Coimbatore Medical College Hospital, during the period from August 2015 to July 2016 under the guidance and supervision of **Dr.A.Dhanasekaran DCH., MD.,** Professor & HOD, Department of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr. MGR Medical University towards the partial fulfilment of the requirement for the award of M.D. Degree (Branch – IV) in Microbiology.

I have not submitted this dissertation on my previous occasion to any University for the award of any degree.

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# *ACKNOWLEDGEMENT*

## ACKNOWLEDGEMENT

I express my deep debt of gratitude to our respectful Dean, **Dr. A. Edwin Joe MD., (F.M), B.L.,** for permitting me to do this study.

I wish to place my deep sense of gratitude and sincere thanks to **Dr. A. Dhanasekaran DCH., MD.,** Professor and Head of the Department of Microbiology, for constant guidance, valuable advice and inspiration throughout my study.

I express my deep sense of gratitude and indebtedness to Professor and Head of the Department of Microbiology **Dr.N. Mythily MD.,** for her the constant encouragement and timely advice given to me during the course of my post-graduation.

I sincerely place my thanks to Associate Professor **Dr.P. Sankar, M.D.,** for his support and encouragement.

I express my sincere thanks to my Assistant Professors **Dr.S.Deepa M.D., Dr.N.Bharathisanthose M.D., Dr.B.Padmini M.D., Dr.C.Ashok Kumar MD.,** and **Dr.R.Radhika MD.,** for their valuable suggestions.

My special thanks to my post graduate colleagues **Dr.M.Banumathy, Dr. R.Senthilkumar,** and other post graduates in the department of Microbiology for their co-operation in completing my study.

I would grossly fail in my duty, if I do not mention here of my subjects who have undergone the pain and discomfort of the investigations during this study.

I take this opportunity to thank all the technical staffs in the Department of Microbiology who gave me their kind co-operation throughout my study.

I affectionately thank my family who are giving their constant support throughout my entire post-graduation course without which this work would not have been successful.

I am thankful to God, who have been with me all throughout my way to reach the destination.

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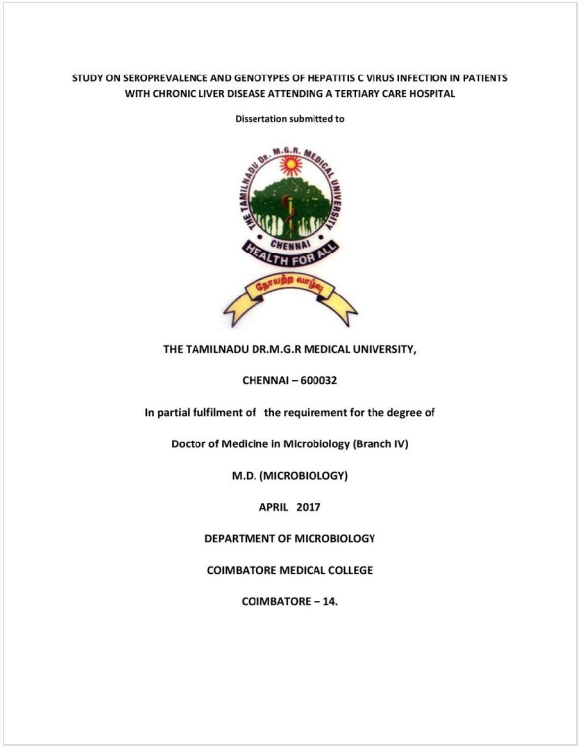


STUDY ON SEROPREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION IN PATIENTS WITH CHRONIC LIVER DISEASE ATTENDING A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY,

CHENNAI – 600032

In partial fulfilment of the requirement for the degree of

Doctor of Medicine in Microbiology (Branch IV)

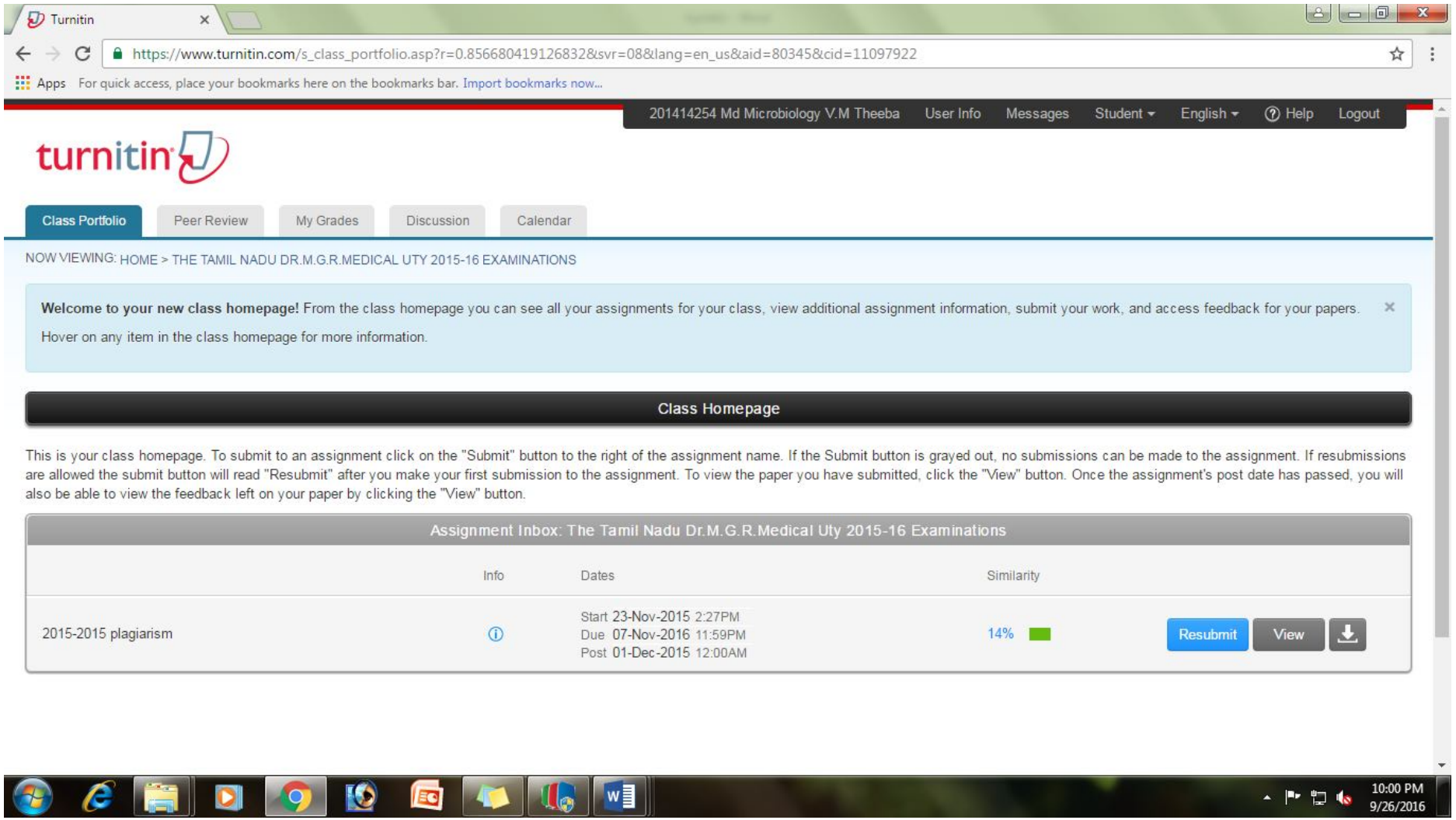
M.D. (MICROBIOLOGY)

APRIL 2017

DEPARTMENT OF MICROBIOLOGY

COIMBATORE MEDICAL COLLEGE

COIMBATORE – 14.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# LIST OF ABBREVIATIONS

| | |
|---|---|
| CDC | Centres for Disease Control and Prevention |
| CHC | Chronic hepatitis C |
| CLD | Chronic Liver Disease |
| CRF | Chronic Renal Failure |
| ELISA | Enzyme Linked Immunosorbent Assay |
| HAART | Highly Active Anti Retroviral Therapy |
| HBV | Hepatitis B Virus |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C Virus |
| HD | Hemodialysis |
| HIV | Human Immunodeficiency Virus |
| IDU | Intravenous Drug Users |
| IFN α | Alpha Interferon |
| MC | Mixed cryoglobulinaemia |
| NANB | NonA NonB |
| NAT | Nucleic acid Amplification Test |
| NC | Negative Control |
| NIH | National Institute of Health |
| PC | Positive Control |
| RIBA | Recombinant Immunoblot Assay |
| RT-PCR | Reverse Transcriptase Polymerised Chain Reaction |
| TNF | Tumour Necrosis Factor |

# *INTRODUCTION*

# INTRODUCTION

Hepatitis C virus is an important cause of chronic hepatitis and primary hepatocellular carcinoma. HCV genotypes can vary in pathogenicity and can have an impact on treatment outcome. Acute infection becomes persistent with long term viremia in 50-85% of infected individuals. Persistent infection with HCV leads to cirrhosis and hepatocellular carcinoma. The high rate of chronicity and lack of successful vaccine makes Hepatitis C virus a serious threat to public health.

According to WHO, Hepatitis C virus is an emerging infection affecting an estimated 130 - 150 million people worldwide and between 3,50,000 and 5,00,000 of them die each year[4]. Chronic infection with Hepatitis C virus is seen in 3% of the world's population [17]. Currently 12.5 million carriers are found in India. HCV infection accounts for about 15-20% of all chronic liver diseases and among 5-10% of Hepatocellular carcinoma cases. It is the most common cause of post transfusion hepatitis. Hepatitis C virus infection is a one of the common cause of liver transplant worldwide.

It is estimated that 4-5 million people are co-infected with HIV and HCV[4]. HCV is one of the important causes for mortality in HIV- positive patients on HAART. Most people infected with HCV are not aware that they are infected; therefore HCV has been called as silent epidemic.

When serological test for HBV and HAV were developed during 1970s it became evident that the majority of transfusion-related hepatitis must be caused by yet another agent called NonA, NonB. Studies in chimpanzees confirmed that NANB hepatitis was transmissible and due to a small lipid enveloped virus. It was first identified by scientists at the CDC and NIH in 1989. It was detected by employing molecular techniques on large volumes of high titer infectious chimpanzee plasma[7]. Key contributors are Harvey Alter, Daniel Bradley and Michael Houghton. Following its discovery, screening for HCV infection has led to a decrease of risk of transfusion-related hepatitis in United States.

Hepatitis C virus belongs to Flaviviridae family, genus Hepacivirus (from the Greek hepar, hepatos means liver). It is a small, single stranded positive sense RNA virus with icosahedral symmetry. The length of genome is approximately 9.6 kilo bases. It is 50-60 nm in size surrounded by an envelope and glycoprotein spikes. The Hepatitis virus does not enter the nucleus. Viral replication takes place in the cytoplasm.

HCV genome is highly mutative, lacks efficient proof reading ability as it replicates. Virions undergo evolution with time and circulate in infected individuals as a population of diverse but closely related variants referred to as “quasispecies”[3]. Mutation occurs in hyper variable region of the genome coding for envelope proteins and escapes from the immune system, at the

same time knocks off innate immunity resulting in chronic persistent infection.

The virus shows considerable genetic diversity. It can be differentiated into 6 genotypes and nearly 100 subtypes based on RNA sequence analysis. Genetic variabilities are recognized as an important factor for the prognosis, monitoring and outcome of HCV mediated chronic liver disease. HCV genotypes have distinct geographical distribution. Genotype 1 is common accounting for 60% global infection. Type 3 is prevalent in South East Asia. Most of the studies in India reported the prevalence of genotype 3 in North and genotype 1 in South.

HCV genotypes differ from each other by 30-35% nucleotide sites. Strains from each subtype vary at <15% of nucleotide sites. Duration of treatment, cure rates, the need for interferon, ribavarin and recently a combination therapy with the new Direct Acting Antivirals (DAA) also base on the genotypes and subtypes. The formulation of treatment strategies using Direct Acting Antiviral requires knowledge of prevalence of HCV genotype. HCV genotype 1,4,5,6 has been reported to show poorer response to conventional antiviral drugs. While up to 80% of the genotypes 2 and 3 can be cured with the standard treatment. HCV 1b is implicated in accelerated progression of chronic liver disease.

The incubation period of Hepatitis C virus averages 6-8weeks. Hepatitis C virus can cause acute or chronic infection. Acute infections are usually asymptomatic or clinically mild. The infection is usually recognized only when it becomes chronic. Spontaneous clearance of infection is unusual in acute Hepatitis C, with nearly 50-90% of the infection becoming chronic. Neutralising antibodies are produced during course of infection, yet the virus mutates to escape from immune system. This leads to persistent infection. Cirrhosis is a significant complication of chronic infection. Hepatitis C infection is an independent risk factor for HCC after development of cirrhosis.

Extrahepatic immunological manifestations, such as cryoglobulinemia, autoimmune thyroiditis, membranoproliferative glomerulonephritis and Rheumatoid factors are a prominent part of hepatitis C infection. Major causes for death in patients with HCV-related cirrhosis are portal hypertension and hepatocellular carcinoma[3].

Blood transfusion, Intravenous drug abuse, unsafe therapeutic injections and health care related procedures are common modes of spread of Hepatitis C virus; Less common routes being vertical and sexual transmission. Major route of Hepatitis C infection is IV drug abuse in developed countries. Common routes of HCV transmission in India are blood transfusion and unsafe therapeutic procedures. This is shown by several studies where the antibody positivity rate for patients who transfused blood before 1995 was

16% and patients who received blood transfusion after 1995 was 6%[47]. In intravenous drug abuse antibody prevalence ranges from 5-93% whereas in hemodialysis patients it ranges from 4.3%-45.2%.

The first step used for the detection of Hepatitis C virus is serological tests. ELISA and the recombinant immunoblot assay are in their third generations with increased sensitivity and more specificity. Serological false negativity in HCV may occur in immuno-compromised individual and during acute phase of infection i.e preseroconversion window period. False positive results can also occur frequently. One possible reason is people who clear infection may remain anti-HCV positive for many years. Serological tests for Hepatitis C Virus cannot differentiate people who had spontaneous resolution from those who are chronically infected. It is necessary to determine presence of virus in the circulation.

The molecular diagnostic techniques are the most sensitive and specific tests. Molecular technique detects HCV RNA and also confirms acute stage of infection. Quantitative assay is necessary for monitoring prognosis. The analysis of Hepatitis C Virus genotypes has become an important factor for planning treatment.

There is a paucity of data available about the prevalence of various HCV genotypes in patients with chronic liver disease in Coimbatore. Considering the importance of Hepatitis C virus infection among chronic

liver disease patients and its associated morbidity and mortality, this study has been undertaken in CMCH, a tertiary care hospital.

The study deals with sero-prevalence and predominant genotypes present among chronic liver disease patients attending medical gastroenterology and allied departments.

# *AIM & OBJECTIVES*

# AIMS AND OBJECTIVES

**AIM:**

To study the prevalence, risk factors of hepatitis C virus infection in chronic liver disease patients attending various clinical departments at CMCH and analyse whether specific genotype is associated with an increased risk of cirrhosis and Hepatocellular carcinoma

**OBJECTIVES:**

1. To screen hepatitis C virus infection in patients with chronic liver disease by ELISA method.
2. To detect HCV RNA by real time RT-PCR.
3. To confirm the Hepatitis C virus sero-positivity by RT- PCR.
4. To identify the genotypes by RT-PCR .
5. To evaluate association of genotypes in patient with chronic liver disease.

# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

## Hepatitis Viruses

Many viruses can produce damage to the liver, most common being primary hepatitis viruses. Yellow fever, Herpes simplex virus, Rubella, Enterovirus and Adenoviruses can also cause hepatitis[1]. Ebstein Barr virus and cytomegalovirus can cause symptomatic hepatitis. The primary hepatitis viruses are diverse group. The alphabet soup of hepatitis is summarized below.

## Hepatitis A virus

Infectious hepatitis is caused by Hepatitis A Virus. It belongs to genus Hepatovirus in the family Picornaviridae. HAV is transmitted principally by faeco-oral route.

## Hepatitis B virus

HBV is the most common type among hepatitis viruses. It belongs to the family Hepadnaviridae under the genus Orthohepadnavirus. It causes serum hepatitis. Infection with HBV can also lead to chronic hepatitis. Hepatic complications are cirrhosis and hepatocellular carcinoma.

## Hepatitis D virus

It is a defective virus which cannot replicate by itself and requires Hepatitis B virus for its survival.

**Hepatitis E virus**

It causes enterically transmitted hepatitis primarily occurring in young adult. It has been assigned to a unique genus Hepevirus under the family Hepeviridae.

**Hepatitis G virus**

HGV was discovered in 1995. It is related to family Flaviviridae, under the genus Pegivirus. It is transmitted through contaminated blood or blood products, or via sexual contact. It is not hepatotropic and does not cause hepatitis.

**Hepatitis virus infection**

**Pathology**

The word 'Hepatitis' means inflammation of the liver. The parenchymal changes are spotty degeneration with necrosis, a diffuse lobular inflammatory reaction, and disruption of liver cell cords which are accompanied by reticuloendothelial cell hyperplasia, periportal infiltration by mononuclear cells and cell degeneration. Localised areas of necrosis and accumulation of macrophages near degenerating hepatocytes are observed.

Histological features of Chronic active hepatitis are inflammation, necrosis and collapse of the normal reticulum framework with bridging between the portal triads or terminal hepatic veins[1].

## Hepatitis C Virus

Ever since its discovery in 1989 as a causative agent of transfusion associated non-A non- B hepatitis[7], HCV has been increasingly recognised as a global health problem. It is a predominant cause of transfusion associated hepatitis and chronic liver disease worldwide, more so in the developing countries. Chronic HCV is associated with wide range of disease ranging from liver cirrhosis end stage liver disease and HCC.

## Epidemiology

### Global burden

HCV has worldwide distribution, affecting persons of all ages, races, gender and regions of the world. HCV accounts for more than 350,000 deaths annually[4], mortality in HCV infection is attributed to liver cirrhosis and hepatocellular carcinoma[2]. Prevalence higher than the global average has been reported from Africa (3.2%) and the Middle East (4.7%) [3]. The Scandinavian countries have prevalence of 0.5% where as in Egypt it is >20%[17].

### Indian scenario

HCV infection is an emerging cause of liver disease in India. Blood transfusion and unsafe injection practices are believed to be two major routes of transmission of HCV in India where about 20 million people are known to have been affected by HCV.

The largest study by Chowdhury and colleagues from west Bengal showed the prevalence of HCV antibody in general population is about 0.87%[6] and among blood donor population 1.8-2.5%[17]. 81% of those who were anti-HCV positive showed the presence of HCV RNA.

**Scenario in Tamilnadu**

A study by V. Gowri et al from Vellore showed overall sero-prevalence in the various group was 0.22%[11.] The sero-prevalence of HCV is 8.2% in liver disease patients[16].

**Hepatitis C Virus**

**Classification and taxonomy:**

HCV has been classified as a member of the family Flaviviridae, along with other related positive stranded RNA viruses. The virus however, is distinct enough to merit classification within a separate genus, Hepacivirus. Closely related genus include the genus the Flavivirus eg., yellow fever virus and dengue viruses and the genus pestivirus eg., bovine viral diarrhea.

**Structure of the virus**

It is a spherical enveloped virus approximately 55nm in diameter. HCV genome is 9.6 kilo bases long[3]. It is a positive sense single standard RNA virus. Its genome has one large open reading frame which accounts for over 95% of the sequence. It encodes for a single large polyprotein,

which is about 3010 amino acids long and it undergoes post transitional modifications to yield 10 viral proteins. Flanking the ORF at both 5' and3' ends are highly conserved untranslated regions (UTR), which mediate crucial steps in viral translation and replication[1].

**Untranslated regions**

The HCV 5'UTR is 341 nucleotides long and contains two overlapping functional regions. The 5' 125 nucleotides are needed for viral replication whereas the remainder of the 5' UTR appear to play an accessory role in this process. It has an overlapping approximately 300 nucleotide long segment, known as "internal ribosomal entry site" (IRES) which directs the cap independent translation of the viral ORF[3].

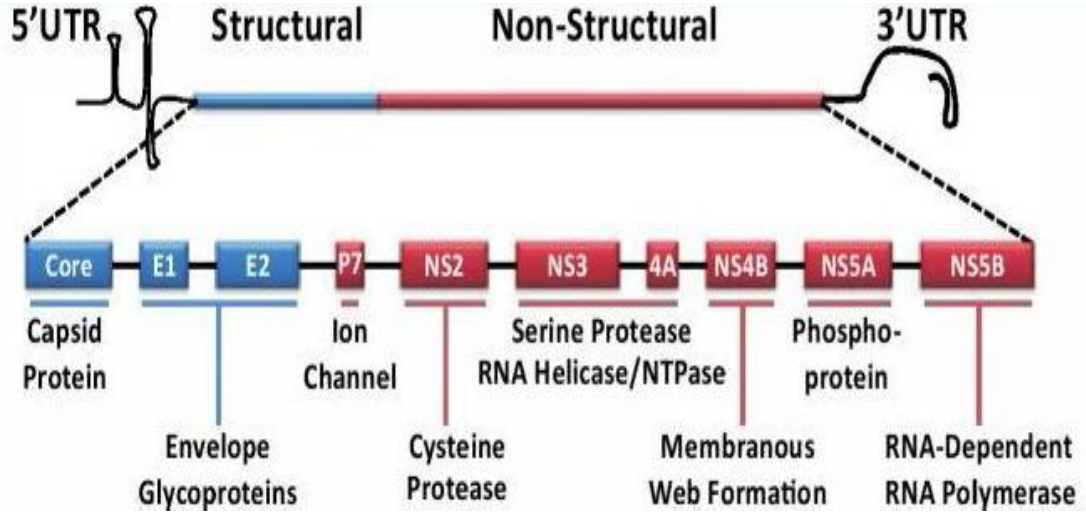
The 3' UTR consists of 30-60 nucleotide segment and highly variable poly-U/UC tract of 50-100 nucleotides. There is a highly conserved 98 - base sequence in the downstream of the poly U/UC Tract designated as 3'X" region. This highly structured 3' terminal 98 base sequence is the most conserved region of HCV genome. The kissing loop interaction between 3'X region with the NS5B coding region and 33 consecutive U residues segment in the poly U/UC tract is essential for viral RNA replication.

Recent work shows that the poly U/UC tract is the principle pathogen associated molecular pattern sensed by human cytoplasmic pattern recognition receptor. The unusual feature of HCV replication that involves the UTRs is

the presence of complimentary sequences for liver specific microRNA 122(miR122). This interaction is found to be necessary for HCV replication, potentially contributing to the hepatotropism of HCV[3].

**Polyprotein**

It is encoded by open reading frame and it is co-translationally processed into atleast 10 proteins. It includes three "structural" proteins: the nucleocapsid protein, core(C) and two envelope proteins (E1 and E2); two proteins that are essential for virion production but are not required for viral RNA replication (p7 and NS2) and five non-structural proteins (NS3, NS4A, NS4B, NS5A, NS5B) which form viral RNA replicase complex.




**Structural proteins**

The structural component of the virus particle is formed by a multifunctional core protein. It is highly basic in nature. The core protein is known to interfere with anti- HCV immune responses through a variety of

mechanisms including NK cell inhibition via upregulation of MHC class 1 expression, inhibition of T cell proliferation via interaction with complement receptor and interaction with the cytoplasmic tail of several cellular receptors belonging to the TNF receptor family.

The core protein is immunogenic, both core protein and its antibody are typically present in the serum of infected individuals.

HCV has two major envelope proteins (E1 andE2) which are produced from HCV polyprotein. The E1and E2 protein are glycosylated with sugar moieties HCV particles assemble and exit the cell by budding into intracytoplasmic vesicles and then follow the secretary pathway for release[3].

A hypervariable segment approximately 30 amino acid residues in length near the amino terminus of E2 is called as HVR 1. It is the most genetically variable segment of the envelope protein. It is assumed to exist as a polypeptide long on the surface of the virion. HVR-1 harbours one or more neutralization epitopes and that is a site of mutations causing immune escape during acute and chronic infection[3].

**P7 and NS2 proteins**

These two proteins may play a role in viral particle assembly or egress from the cell. The protein is produced by cleavage of E2 near its carboxyl terminus. This is a small 63aa hydrophobic polypeptide which appears to be capable of forming a voltage gated ion channel. Its activity

is required for the production of infectious virions, inhibited in vitro by amantadine and long alkyl chain imino-sugar derivatives, representing a possible therapeutic target[3].

The NS2 protein is a membrane associated dimeric cysteine protease with two composite active sites which mediate cleavage at the NS2/NS3. The transmembrane and protease domain structures of NS2 are needed for the formation of infectious particles in cell culture whereas the protease activity is not.

**Non-structural proteins**

These proteins are required for RNA replication which occurs in a membrane associated replicase complex within the cytoplasm. The NS3 protein possesses serine protease activity in its amino terminal end and an RNA helicase with NTPase activity in its carboxy terminus. Its activity depends upon zinc. It is responsible for the NS3/NS4A cis cleavage and cleavages of NS4A/NS4B, NS$B/NS5A, NS5A/NS5B. TheNS4 protein acts as cofactor for the NS3 protease. An amino terminal segments of the protein binds the NS3/NS$A complex to intracellular membrane, while NS4A complex also interacts with NS5A.

NS4B is a hydrophobic membrane associated protein, which mediates modifications of the ER membrane that occur in association with replicase assembly and also inhibits normal ER-to- Golgi secretory pathways.

NS5A is a membrane bound anchored RNA binding phosphoprotein that appears to play role in RNA replication. NS5B is a membrane bound protein that contains a Gly-Asp-Asp motif characteristic of RNA dependent RNA polymerase and is considered as the catalytic core of the replicase complex. As with a enzymatic activity of NS3 protein, the NS5B RNA polymerase has proven as a useful target for drug development with nucleotide analogue and non-nucleotide small molecule inhibitor as well as cyclosporine A analogue.

**Replication**

Life cycle of HCV begins with attachment and internalisation of virus into the host cell. It is mediated by viral envelope glycoproteins E1 and E2. A number of host cellular receptor such as CD81, LDL receptor, and human scavenger receptor SR-B1, DC- SIGN, claudin-1 and occludin are believed to be necessary for this process.

After attachment and entry, uncoating of the nucleocapsid occurs, which leads to release of the viral RNA into host cytoplasm. As it is a positive stranded RNA it act as messenger RNA and translation of the polyprotein is initiated following ribosomal mediated binding mediated by the IRES domain.

This is followed by a number of cleavages of the polyprotein by both cellular and viral proteases which results in the formation of various structural

and non-structural proteins. After cleavage the core protein stays in cytoplasm while E1 and E2 are secreted in to the endoplasmic reticulum. The non-structural proteins assemble to form a membrane bound replication complex.

The viral NS5B RNA dependent RNA polymerase (RdRp) facilitates the synthesis of a negative stranded intermediate. This consequently serves as a template for synthesis of positive stranded RNA. This RNA, core protein, E1 and E2 proteins gets packaged into new viral particles. After maturation and assembly, newly produced virions are released from the host cell through the secretary pathway.

**Genetic diversity**

**Quasispecies variation**

The high turnover of virion in the absence of proofreading ability by NS5B RNA polymerase and tolerance of many genomic regions for multiple nucleotides resulting in the rapid accumulation of viral mutations. Accumulation of a multitude of closely related but distinct HCV variants within an infected individual are known as quasispecies.

Viral RNAs containing spontaneous mutation within the HVR - 1 segment of the E2 protein may be favoured for survival in the host when they reduce the binding of pre-existing neutralising antibodies to the envelope. Quasispecies variation occurs in a single individual[3]. This heterogeneity of the viral population may rapidly select treatment resistant clones, thus

possibly reducing treatment efficiency of the new direct acting antiviral drugs recently approved for treating HCV infection.

**HCV Genotypes**

Tremendous genetic heterogeneity and variation among sequences of HCV isolated from different individuals that has led to their classification into genotypes and subtypes. Genotyping and subtyping of HCV is useful for understanding of epidemiology, vaccine development, clinical management and therapeutic measures against chronic HCV infection.

Genotyping is done by sequencing either the 5'UTR/core, NS3 or of the NS5b region of HCV genome. Phylogenetic evaluation of HCV sequences recovered from different geographic zones suggests that there are 6 genotypes or clades. Diversity of the genotypes at the nucleotide level is estimated to be about 30%.

Within individual HCV genotypes strains can be further grouped into subtypes that generally share 75%-85% nucleotide identity within the core E1 and NS5B regions of genome. Alteration in the rate of HCV multiplication, response to interferon therapy, or pathogenicity of the virus due to differential activity of HCV proteins is because of the difference in nucleotide sequence.

The quasispecies variations that exist within a single person generally have 91-99% identity in these regions[3]. This genetic diversity is independent

of differences in clinical disease although variations exist in response to antiviral therapy according to viral genotypes[1].

Recently, studies emphasised clinical importance of typing and subtyping. Genotype 1 in particular responds poorly to IFN-alpha, while genotypes 2, 3 can be treated favourably. Studies on Japanese patients showed that the outcome of HCV subtype 1b infection treated with alpha interferon therapy is correlated with genetic diversity in the NS5A gene. Infection with genotype 1 may progress rapidly to cirrhosis and HCC compared to genotype 2 and 3.

**Viral tropism**

HCV multiplies inside the liver cells, and the liver specific expression of miR122 may contribute to this specificity. Some studies observed the presence of negative strand HCV RNA in T cells, B cells and monocytes especially in patients affected by CHC.

**Global distribution of HCV genotypes**

Genotype 1 found to be the commonest genotype with a worldwide distribution in USA and northern Europe[29]. Genotypes 2 and 3 are also found worldwide, with a higher prevalence in Europe, North America and Japan[30]. Genotype 3 infection is common in Southeast Asia and in the Indian subcontinent and is also prevalent in intravenous drug users in the USA & Europe. Genotype 4 infections are mainly present in Northern Africa and

Middle Eastern Countries. Genotype 5 appears to be restricted to South Africa. Genotype 6 is restricted to intravenous drug users in Southeast Asia and more recently in Australia[30, 31, 32].

**Distribution of HCV genotypes in India**

There are a few studies which have attempted to establish the distribution of HCV genotypes in the country.

In the largest such study by Christdas et al.[12], spanning over a decade 2002-2012 and including 451 patients from various parts of the Indian subcontinent, genotype 3 was found to be the most predominant 63.85% followed by genotype 1,4,6 (25.72%, 7.5% and 2.7% ). Genotype 2 was found in only one patient from Northeast India and genotype 5 not detected till now.

Genotype 1 was commoner in South India while genotype 3 was more prevalent in East and North-east parts of the country. Genotypes 4 and 6 appeared to be restricted geographically to the southern and north eastern parts of the country respectively, which has been published previously as well[33, 34]. Recombinant strains of genotype 1 and 2 were isolated from two patients.

In another study on 398 patients from North and Central India by Hissar et al, the findings were similar. Genotype 3 was the commonest genotype, seen in 80.2% patients, followed by genotype 1 in 13.1% patients[35].

Genotypes 4(3%) and 2(2.5%) were rare. There were no cases of genotypes 5 and 6 infections. Five patients showed mixed genotype infection.

The study by Sompal singh et al on chronic hepatitis C cases in north India showed that Genotype 3 was found to be the widespread genotype [25].

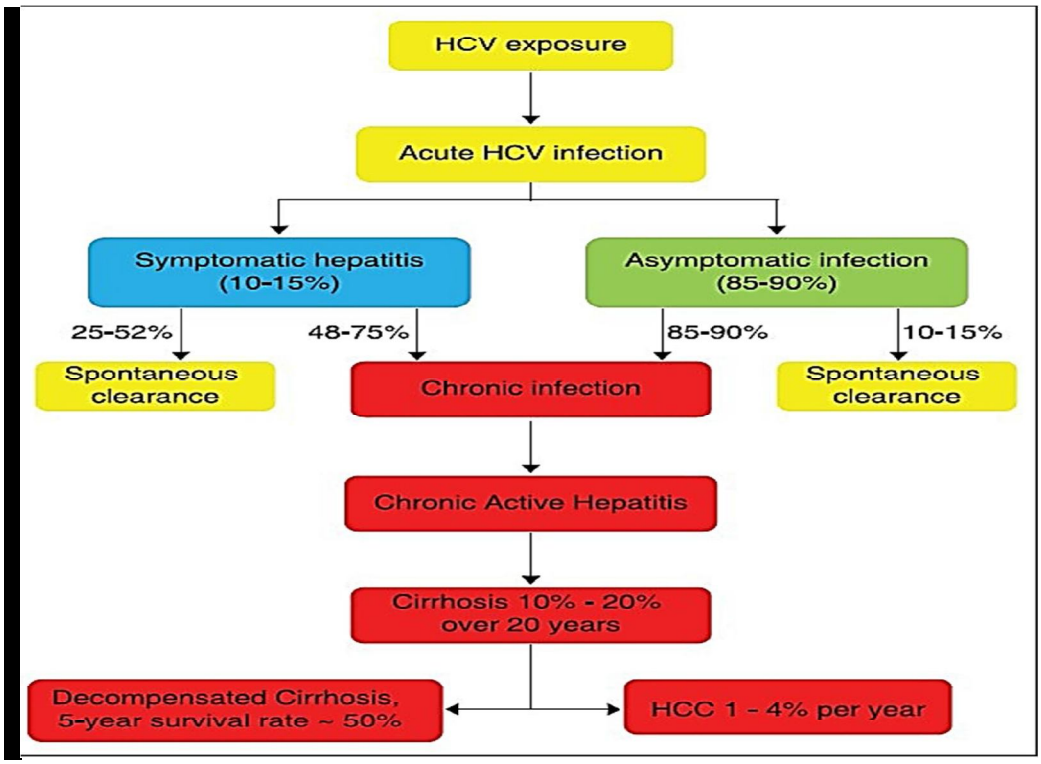
**Natural History and Pathogenesis**

HCV RNA can be detected in plasma within days of exposure, often 1-4weeks before liver enzyme levels rises. Viremia reaches maximum in the first 8 to 12wks of infection then falls to lower levels and persists. In some instances plasma RNA becomes undetectable in the first few months and remains undetectable indefinitely (viral clearance).

In other instances, Viremia is inconsistently detected early and a stable pattern of recovery or persistence is not evident for more than 6 month. In some cases intermittent Viremia may reflect reinfection which has been observed in active intravenous users. In other cases rebounding Viremia may indicate escape from an initially successful viral response.

Overall viremia persists in 50-85% of acutely infected persons. HCV infection more often persists in African, American people than whites and in person infected with HIV than immunocompetent persons. Persons who develop clinical symptoms are more likely to clear infection. This correlates with the more vigorous immune response in them. Noththless it is difficult

to define the immunological mechanisms of HCV persistence and their genetic determinants.




**Acute hepatitis c and spontaneous clearance**

Acute hepatitis can be caused by HCV. Acute infection with HCV is mostly asymptomatic. It is possible to detect HCV RNA in most of the patients within 1 to 2 weeks & is followed by an increase in liver enzymes by 2-8 weeks. Symptoms of acute hepatitis develop within 3 to 12 weeks of exposure to the virus, in about 25 to 30% of patients. However anti-HCV is not reliable in the diagnosis of acute infection as up to about thirty percent of cases will test negative at the onset of symptoms because of

delayed sero-conversion. Almost all patients will eventually develop anti HCV whereas the titre will be low in the context of immunosuppression[9].

**Chronic HCV infection and progression of fibrosis**

Persistence of HCV RNA for more than 6 months after onset of infection defines chronic hepatitis C. Age at acquisition of infection, sex, race, immune status of the patient, co-infection, along with other host and viral factors influence chronicity of the infection[8]. The early phase of the infection is marked by appearance of HCV RNA, followed by rise in serum transaminases.

It must be noted that in the time period of evolution from acute hepatitis to chronic hepatitis, HCV RNA and enzyme levels can vary remarkably. Once the infection gets persistent, viral load tends to stabilise. Spontaneous resolution of chronic infection is unusual. Histological finding is the main criterion for assessing severity and disease progression[26].

Fatigue, abdominal discomfort, nausea, and poor appetite are the most common symptoms seen[9]. The disease may remain clinically silent for decades. However, hepatocellular inflammation and fibrosis continues, leading to progressive liver disease. The rate of progression of the disease is again determined by a multitude of modifiable and non-modifiable factors.

Progressive hepatic fibrosis may lead to cirrhosis and decompensated liver disease. Such cases have a high risk of developing hepatocellular

carcinoma, with 1 to 4% of patients developing this complication each year[10]. It usually takes more than two decades of infection for these long term complication to develop, unless accelerated by coexistent factors. Prognosis of patients with cirrhosis is influenced by the onset of complications that occur at a yearly rate of about 5-7% patients.

**Extrahepatic manifestations**

Seventy four percent of patients have reported Extrahepatic manifestations. Extrahepatic manifestations are Cryoglobulinemia, Membranoproliferative glomerulonephritis, Raynaud syndrome, Sjögren syndrome, Necrotizing cutaneous vasculitis, Non-Hodgkin lymphoma.

Patrice Cacoub et al study showed that Mixed cryoglobulin, RF activity, and Antinuclear antibody, Anticardiolipin antibody, Anti-thyroid antibody and Anti-smooth muscle antibodies are most frequently seen immunological abnormality in HCV[27].

A minimum of one immunologic abnormality can be seen in up to fifty three percent of HCV patients. Signs and symptoms of a connective tissue disease (except for mixed cryoglobulinaemia) are not caused by these autoantibodies. Autoantibody synthesis in these cases is due to the HCV-induced over activation and proliferation of B lymphocytes.

**Mixed cryoglobulinaemia**

HCV infection is the cause of Mixed Cryoglobulinaemia in roughly about eighty percent of the patients.

**Sjögren syndrome**

HCV infection has long been suspected as a potential cause of SS. There is a possible relation between hepatitis C virus and Sjogren syndrome as it can be excreted in saliva.

**Disease progression**

The major pathological outcome of chronic HCV infection is development of hepatic fibrosis. It usually followed by progression to cirrhosis and then to hepatocellular carcinoma. This complication usually takes more than twenty years after the onset of infection. It has been estimated that probability of cirrhosis occurring within twenty yrs. of infection is 5%-25%[3]. HCV infection is usually asymptomatic. It is difficult to assess rate of progression of fibrosis prior to clinical manifestion.

Studies showed that mortality was slightly more in patients with post transfusion hepatitis (3%) than control group (1.5%) [3].

The leading environmental determinant appears to be alcohol ingestion. Excessive alcohol consumption and HCV infection independently can cause cirrhosis; Exposure to both will have synergistic effect. Alcohol and HCV

infection may cause microvesicular steatosis, suggesting a common pathway involving mitochondrial injury.

Co-infection with HBV can also accelerate disease progression. High level of HCV viremia is found in HIV which can lead to accelerated progression of liver disease. Schistosomiasis co infection is associated with much more rapid progression of HCV mediated fibrosis in Egypt[90]. Accelerated progression of liver disease is also expected in immunosuppression associated with agammoglobulinemia and organ transplant.

**Immune response to HCV Infection**

HCV infection triggers sequence of intracellular events that lead to the development of an antiviral state in the infected cell and the surrounding tissue. Following viral entry into the host, pathogen associated molecular patterns in the viral genome are recognised by PAMP receptors expressed on the host cell, initiating the host immune response. Retinoic acid inducible gene 1(RIG-I) and Toll-like receptor 3 are two major receptor pathways triggered by HCV RNA. This subsequently stimulates interferon stimulated genes inducing endogenous interferon production, and thus building the initial antiviral defence[36]. For strategies to evade host immune response, it is the balance between the two which determines progression of the disease.

## Innate Immune response

### Interferons and Interferons stimulated genes

The first response to HCV infection is by the production of endogenous Interferons by the infected liver cells. This begins With Toll like receptor -3 and RIG- 1 mediated sensing of HCV RNA, which through various mediators leads to signalling of IFN regulatory factor 3. This induces the transcription of IFN- β, creating an antiviral state in infected and uninfected neighbouring cells via paracrine effects, limiting cell to cell spread. Interferon-beta binds to IFN-α/β receptor activating the JAK/STAT pathway. This causes stimulation of Interferon stimulated genes, which have different antiviral properties, such as degradation of viral nucelic acid, inhibition of translation and destabilisation of secondary structures of viral RNA.

Some pattern recognition and signalling molecules like RIG-1 are also ISGs, whose levels markedly increase from low basal level, increasing the sensitivity of downstream signalling in infected tissues and promoting IFN and ISG production. Another ISG, IRF7 stimulates IFN α production, thus diversifying the IFN response and providing a positive feedback to ISG expression[36, 37]. The current treatment for HCV focuses on the IFN α component of immune response.

## Adaptive immune response

### Humoral immunity

Within months of infection, antibodies are detectable in blood to multiple recombinant antigens that correspond to structural and non-structural protein genes. These antibodies are neutralising in nature, differing in their mechanism of neutralising. The antibodies are isolate specific and together with CD8 cells contribute to the evolution of HCV quasispecies by exerting selection pressure. Lack of temporal relation of these antibodies to viral recovery and demonstration of HCV clearance in individual with agammoglobulinemia led to the belief that humoral immune response was neither necessary nor sufficient for viral clearance[37, 38].

However recent studies showed the role of the neutralising antibodies in outcome of the disease. Early and rapid production of antibodies may lead to spontaneous resolution of infection. In case of chronic infection where antibodies are either absent or very low in titre in early phase of the infection influencing the outcome of the disease[49].

### Cellular immunity

HCV specific CD8 and CD4 T cell responses were known for being critical for HCV clearance. Functional CD4 response is an essential factor which decides the fate of Hepatitis C Virus infection by production of Interleukin-2 and Interferon-γ. Vigorous proliferation of HCV specific

CD4 cells is seen in individuals who clear the virus and impaired or weak response is seen in those who progress to chronic disease[39].

On the other hand HCV specific CD8 cells are detectable in cases of acute infection irrespective of virological outcome. In acute infection some CD8 cells show a "stunned" phenotype and are unable to produce IFN-γ. However, as CD4 T cell responses develop and viraemia declines, this dysfunction resolves and memory cells become detectable[40].

In cases of recovery, durable populations of memory T cells are seen. In chronic infections, persistent antigenic stimulation along with impaired CD4 T cell function leads to CD8 T cell exhaustion. This state is marked by loss of CD8 T cell cytotoxic functions, TNF-α production, and eventually IFN- γ production along with dysfunctional memory T cells as is often the case in chronic HCV infection[37].

### 4.2.3. Evasion of Adoptive Immune Response by HCV

A lot of theories for persistence of HCV infection are hypothesized, but the following three mechanisms have substantial experimental support[38].

1. Mutational escape of viral epitopes

The error prone nature of the viral polymerase generates viral variants capable of evading cytotoxic T cells and neutralizing antibodies.

2. Functional anergy of CD8 T cells

HCV specific CD8 T cells may be anergic or functionally impaired in chronic infections.

3. Regulatory T cell populations

Intrahepatic CD8 T cell populations producing IL-10 are known to occur in chronic infections. IL-10 impairs production of IFN and down regulates effector T cell responses.

**Mechanism of persistence**

Confection with HIV and schitosomiasis have been associated with viral persistence which corresponds with a diminished CD4 lymphocyte response[3]. The highly glycosylated nature of the viral envelope may protect it against antibody mediated neutralisation. The envelope may have evolved a flexible structure that serves as an immunological decoy and protects an otherwise vulnerable, conserved receptor – binding ligand from antibody attack. The virus may downregulate replication to a level that is too low disrupting cellular homeostasis, limiting the amount of viral PAMPs and antigens produced. HCV sequence variation and immune escape from both T cell and B cell may also contribute to viral persistence.

Mutation within the amino acid sequence of a critical epitope might permit a new quasispecies variant to escape an immune response. In several

studies acutely infected persons who developed persistent infection had a more complex quasispecies.

**Factors affecting disease progression**

Many factors are associated with increase in progression of the disease. These include predilection for male, elderly age, overweight, intake of alcohol and co-infection of HIV and HBV.

**HCV-HIV co-infection**

HCV infection is found more frequently in HIV infected persons than in general population because of a common mode of transmission. The prevalence of HCV- HIV co-infection also markedly varies from 50-93% in intravenous drug abuse and 10% in homosexual men depending on the route of transmission. Prevalence of HCV-HIV co-infection is 3.02% in Andhra Pradesh, 2.2% in Tamil Nadu , 1.6% in Lucknow and 1.06% in Vellore, as reported by several studies[11,13,14,15].

The predominant age group affected was 41-50years and the most prevalent genotype seen in HIV-HCV co-infection was genotype 1b[13]. Higher rate of HCV RNA was found in HIV, HCV co-infection.

**HCV - HBV co-infection**

Co-infection of HBV in the proportion of HCV-infected people can have an effect on progression of hepatic disease. A meta-analysis showed that HBV-HCV co-infection more likely to cause hepatocellular carcinoma.

**Modes of transmission**

The common routes of transmission are blood transmission, injection abuse, unsafe therapeutic interventions and health care related infections. Sexual transmission and vertical transmission are less common routes of transmission.

**Blood transmission**

Blood transmission is the major mode of transmission. Due to the mandatory screening, HCV transmission risk is low in developed countries. In developing countries blood transfusion is a common cause of acquiring HCV infection. Blood transfusion allows large quantities virions to enter into the blood. Studies have shown that prevalence of HCV is below 2% in voluntary donors. In India mandatory HCV screening of blood and blood products was introduced in 2002. Few cases of infected blood donor may be missed if serological tests alone are used.

V. Gowri et al study in Vellore showed sero-prevalence of HCV among voluntary blood donor is 0.13%[11].

Chandrasekaran S et al study in Madurai showed sero-prevalence of HCV among voluntary blood donor is 0.75%[41].

**Unsafe therapeutic injections**

In resource limited settings, where supply of sterile syringes may not be available and injection are administered by non-medical personnel outside

the hospital. Persons who receive multiple contaminated injections over a period of time increases the chance of acquiring Hepatitis C Virus infection.

**Intravenous drug use**

It is a common route of HCV transmission in developed countries. Highest sero-prevalence in the middle age group seen in United States and Australia, where the IVDs have been the predominant mode of spread for nearly 3 decades which constitutes sixty eight percent and eighty percent of current infections respectively.

Lopamundra Ray saraswati et al study observed that the prevalence of HCV in 53.7 %, and co-infection of HIV in 19.6 % male intravenous drug abusers in Delhi in 2012[19].

In a study by Shruti H. Mehta et al study conducted at YRGCARE Chennai 1158 Intravenous drug users were screened for HCV infection. The study reported a prevalence of 55%. Sharing partners are necessary for maintaining transmission of HCV than other any other blood-borne viruses. HCV is ten times as infectious as HIV, per unit of blood evidence shows that, after drying at room temperature, HCV can remain infectious for about six weeks in dried blood spots. This might be the reason for the constant spread of HCV among IVD's. This could be due to unsafe injection practices like sharing equipment, drawing from a common container and injecting with used needles.

**Health care associated infection**

Occupational transmission of HCV infection occurs in health-care workers who have sustained a contaminated needle stick injury and observed risk of transmission in these circumstances are as low as 0·3%.

**Haemodialysis**

Patients undergoing haemodialysis (HD) have a higher risk of acquiring HCV. In these patients infection more likely to become chronic. This may be responsible for transmission of HCV in dialysis centres, contributing to the increased prevalence of HCV among haemodialysis cases.

Jaiswal et al in a study from 1992 to 2000 reported prevalence of 30%[21].

Pragati chigurupati et al showed a prevalence of 23.5% in HCV infection among the hemodialysis patients[22].

Sudrandrakumar et al's study in Coimbatore showed the patients on hemodialysis had 12.4% positivity for anti- HCV in dialysis unit. Further, the study demonstrated that the duration of haemodialysis and undergoing dialysis at more than one centre were the important risk factors for acquiring HCV infection[42].

HCV prevalence in HD patients was found to be 4.3% in Delhi and 1.11% in Mangalore. Gomes M et al study observed the prevalence of anti-HCV was 13.3% in patients who had HD for <1 year in comparison to

69.9% in patients who had HD for >10 years -indicating that the duration of HD is related to a higher risk of developing HCV infection.

Nosocomial transmission, prolonged vascular access, risk of exposure to infected patients, contaminated equipment and sharing of multi-dose heparin vials are some factors responsible for the high risk in HD patients.

The clinical outcome HCV in haemodialysis patients vary from the general population. Hemodialysis patients will have a milder disease with lower liver enzymes and viral levels. The “silent” clinical course is results in slower disease progression and a lower frequency of cirrhosis and hepatocellular carcinoma. Possible reasons are impaired surveillance leading to a less aggressive antibody response to the virus and intradialytic release of “hepatoprotective” cytokines

**Sexual transmission**

Sex transmission has been identified as minor risk factors for HCV transmission.. The prevalence of HCV infection in individuals with STDs in in South India was 6%.

**Vertical transmission**

Mother –child transmission is estimated to occur in about 2·7–8·4% of babies born to HCV infected mothers. New-born born to HIV/HCV co-infected mothers have higher risk of acquiring HCV infection[44].

Kumar et al study reported a prevalence of 1.03 % in antenatal population[43] while the results of other studies at Shimla and Vellore reported no (0%) prevalence. The timing of transmission is not known. The confirmation of HCV transmission is based on detection of viral RNA or the persistence of antibodies after 18 months of age. HCV RNA has been detected in breast milk.

**Diagnosis of HCV infection**

Testing for infection is mainly done for a clinical diagnosis of liver disease in symptomatic individuals and as a part of mandatory screening in blood banks for all donors. It is also advisable to screen people who are at a high risk for the HCV infection. Guidelines recommend HCV screening should be done in persons with HIV infection, haemophilia, haemodialysis, drug abuse, recipients of blood transfusion organ transplant before 1992; children born to HCV infected mothers and health care workers[45]. Diagnostic tests are broadly classified into serological assays (indirect tests), detection of HCV RNA and core antigen (direct tests).

**Serology**

Detection of HCV specific antibodies is an indicator of infection, not immunity. Current serological assays are able to detect HCV 5- 8 weeks after the onset of infection. In patients who clear infection spontaneously, HCV antibody may remain throughout life, decrease slightly and gradually

disappear after several years. Anti-HCV persists indefinitely in patients who develop chronic infection, except in cases of profound immunosuppression.

Immuo-assays are based on enzymatic reactions like ELISA or light emission like CLIA. Different generations of HCV ELISA detecting antibodies to different recombinant polypeptides have been developed. The first generation ELISA target a part of NS4 region of HCV genome. The second generation target a part of NS4 and protein derived from NS3 and a part of core(C-22). The third generation ELISA detects antibodies against NS5 as well. It has a high sensitivity of about 97%. Recombinant immunoblot assay (RIBA) can be used to identify the specific antibodies against individual HCV antigens.

Serological assays have following drawbacks:

- False negativity in the window period between entry of virus and the production of antibodies.
- False positivity results may due to presence of non specific immunoglobulins that can bind with HCV antigens.
- False-negativity can occur in immunosuppressed people or in those who are undergoing haemodialysis.

According to CDC person be considered to have serologic evidence of HCV infection if a positive result of anti-HCV screening is confirmed by

positive results of a further test, using either a RIBA or NAT to detect HCV RNA. Rapid immunoassay helps I in rapid detection of antibody.

**HCV core Antigen detection**

The Core antigen can be present in infected patients, and its levels are proportional to HCV-RNA. Cost of Core assays is less than molecular tests [46]. They could be used as an alternative to HCV-RNA assays in the following conditions:

- To differentiate active infection from resolved.
- To detect antigen in the window period.
- Detecting infection in high-risk seronegative individuals.

**Molecular assays**

**Detection of viral nucleic acid**

Detection of HCV RNA is necessary to establish active infection, either acute or chronic, as well as for monitoring the patients on treatment. real time RT-PCR, transcription mediated amplification (TMA), and branched DNA testing can be used. Assays that detect nucleic acids can be qualitative or quantitative.

Most sensitive test for detection of viral load is quantitative PCR. Most precise method for quantitation is branched-chain DNA test. Molecular assays have excellent specificities 98 to 99% and sensitivity varying from 10 to 50 IU/ml[45].

While qualitative methods may be sufficient for screening in blood banks, quantitative assays are used to measure the baseline viral load prior to initiation of therapy, and then at specified time points for monitoring the treatment response during the course of therapy.

WHO recommended the use of standard international units (IU) for the measurement of the viral RNA instead of viral copies. During acute hepatitis, the delay in the appearance of anti-HCV hampers acute phase diagnosis. The early detection of HCV RNA in peripheral blood confirms the diagnosis and opens up therapeutic possibilities. In chronic hepatitis, the diagnosis of sero-negative forms may only be resolved by PCR. Moreover, the presence of HCV RNA in peripheral blood represents the only marker of ongoing viral replication and coincides with the hepatic damage. During treatment with interferon, the follow up of HCV RNA sequences makes it possible to monitor its efficacy.

The search for HCV RNA sequences directly in liver tissue shows that HCV may multiply in the hepatocytes in the absence of virus in the circulation. The demonstration of HCV RNA in the hepatocytes in the liver transplanted patients is essential for etiological diagnosis. Epidemiological study using PCR is a major tool for documenting vertical transmission between mother and child. PCR is important for the analysis of the HCV genome.

**Viral genotyping**

Determination of genotype of the infecting virus is necessary to assess the probability of response. It also helps in deciding the duration of treatment. Genotyping can be done by sequencing either the 5'UTR/core, NS3 or the NS5b region of HCV genome. A number of assays are available for the same and include real time PCR with genotype specific probes and primers reverse hybridization of PCR products into genotype specific probes coated on solid supports (line probe assays), PCR-RFLP, where the PCR products are digested with restriction enzymes, to obtain fragments of varying length depending on the genotype.

**Liver function Tests:**

Liver function tests are done to assess liver function. The tests done are aminotransferases, alkaline phospatase, serum bilurubin, serum proteins, prothrombin time and APTT.

Trea**tment of Hepatitis C infection**

Hepatitis C is a severe infection causing considerable morbidity and mortality. The main concern is progression to liver cirrhosis and its accompanying complication. Patients with chronic infection are also at risk of extrahepatic manifestations even in the absence of progressive fibrosis, some of which may be severe.

Antiviral treatment is necessary to prevent both hepatic as well as extrahepatic sequelae of infection. Virological care, marked by sustained lack of viraemia six months after completion of treatment, associated with decrease in liver inflammation, as evidenced by stabilised enzyme levels and decreased rate of progression of fibrosis indicates good prognosis.

Timely treatment has been shown to decrease the development of end stage liver disease, need for liver transplantation, hepatocellular carcinoma rates and liver related morbidity. The Presence of non-modifiable factors, genotype 1, a heavy viral load, overweight, ethnic race, older age, and fibrosis, indicates poor therapeutic response. Sustained virological response helps is assessing therapeutic response.

The primary aims of anti-HCV therapy for chronic hepatitis C cases are prevention of progression to cirrhosis and development of HCC. A combination of pegylated interferon and ribavirin cures HCV in about 40% to 50% of untreated patients with genotype 1b. In patients achieving SVR, in interferon therapy has improved liver fibrosis.

However, only limited numbers of patients show beneficial antiviral effects of Interferon based therapy. The effect depends on the patient's genetic background, presence of hepatic fibrosis, age, HIV co-infection, and other factors. In addition, IFN-based therapy has some adverse effects that may lead to poor drug adherence or treatment discontinuation.

Recently, direct-acting antiviral (DAA) regimens have been approved for treatment. The first-generation protease inhibitors telaprevir (TVR) and boceprevir (BOC) were approved as DAA combination therapy with Pegylated Interferon and Ribavarin. Although triple therapy achieves a higher SVR rate than does conventional IFN-based therapy, treatment is associated with severe adverse effects. Due to the development of new DAAs with better safety and stronger antiviral effects, it is expected that almost all patients with HCV infection will achieve SVR.

**HCV vaccine**

Development of HCV vaccine is most challenging and several factors that hinder the development of vaccine are:

1. Presence of genetic diversity among isolates (six genotypes, >100 subtypes).
2. Production of quasispecies within an individual due to mutation in the HVR1 region of structural E2 gene.
3. Poorly defined immunological correlates of protection and finally,
4. Inability to propagate in culture to isolate the virus.

# MATERIALS & METHODS

# MATERIAL AND METHODS

## Study design

This prospective study was conducted in the Department of Microbiology, Coimbatore Medical College, Coimbatore over a period of 12 months from August 2015 to July 2016.

Blood samples were collected from 200 patients admitted with chronic liver disease for detecting Hepatitis C virus infection in various clinical departments of Coimbatore Medical College hospital.

## Ethic approval

The approval for this study was obtained from the ethical committee prior to its conduct. Informed consent was obtained from the patients/ guardian of the patients

## Inclusion criteria:

- Patient with chronic hepatitis
- Fulminant hepatitis
- Hepatic failure
- Cirrhosis of Liver
- Hepatocellular carcinoma
- Persistently elevated Liver enzymes, Serum bilirubin > 6 months

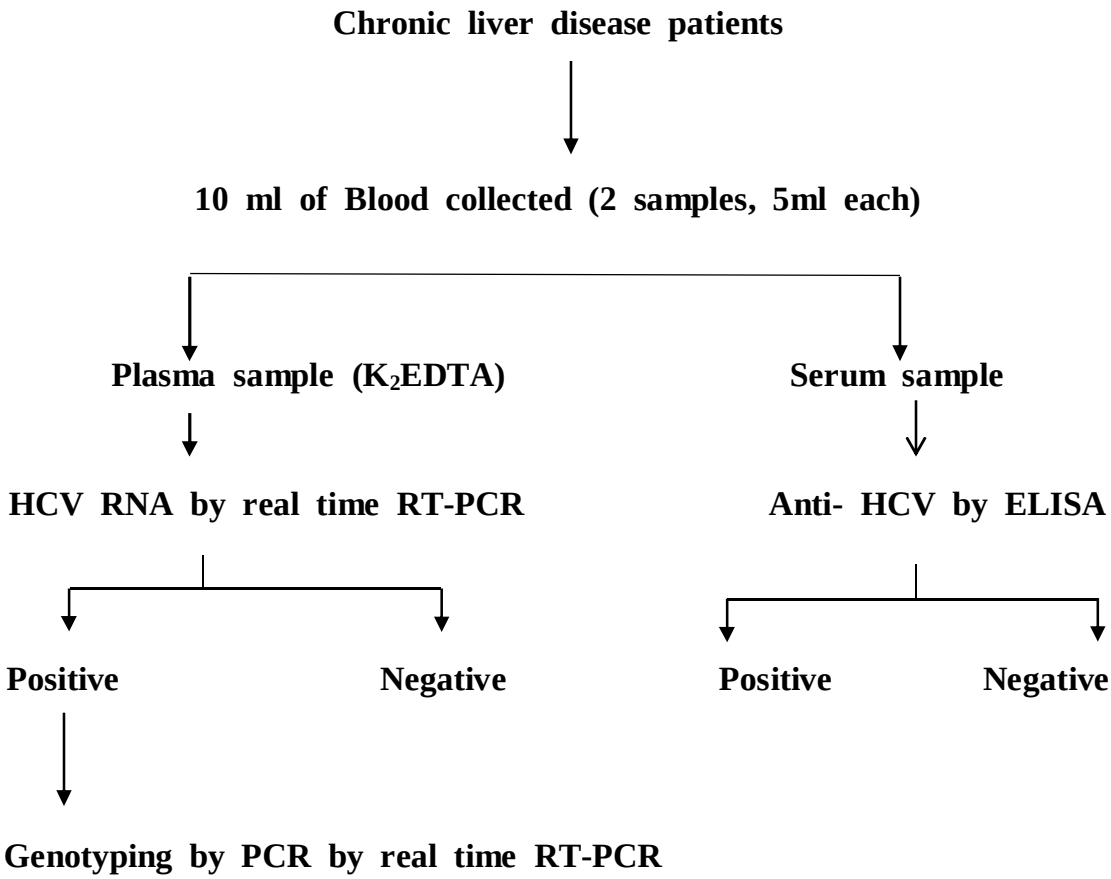
**Exclusion criteria**

- Patients with acute hepatitis
- Auto immune diseases of liver
- Inborn errors of metabolism
- Systemic diseases affecting liver function
- Hepatic congestion due to cardiac failure
- Drug induced hepatic dysfunction

The patient name, age, sex, address, Inpatient/outpatient number and detailed clinical history were noted. General and systemic examination was carried out.

Ten ml of blood was withdrawn aseptically by venepuncture. The 5ml blood sample for serum was transferred to plain tube (clot activator) for Anti HCV ELISA. The 5ml blood sample for plasma was collected in a vacutainer tubes containing $K_2$EDTA and the tube was centrifuged at 3000rpm for 10 minutes for separation of plasma. This was stored at - 20°C for PCR. ELISA test for HCV antibody is performed using Hepascan kit as per manufacturer instruction and samples is tested for detection of HCV-RNA by reverse transcription and real time polymerase chain reaction (RT-PCR). Genotyping of the positive samples were done out by real time RT-PCR.

## METHODOLOGY



**Procedure for Anti HCV ELISA**

Detection of HCV infection usually begins with serological testing to detect anti HCV antibodies.

**The HCV ELISA**

The HCV ELISA TEST (Hepa-scan) is an indirect antibody Enzyme immuno absorbent assay for the invitro detection of antibodies to HCV in human plasma and serum. It is a third generation test, uses a greater range of antigens from core, NS3, NS4 and NS5 regions of the virus to selectively detect all sub types of hepatitis virus in human plasma or serum with high degree of sensitivity and specificity.

**Requirements:**

- Microtiter plate
- Dilution buffer
- Wash solution
- Horse radish peroxidase conjugate
- Tetra methyl benzidine substrate
- Tetra methyl benzidine diluent
- Stop solution
- Positive control
- Negative control
- Micropipettes

- Plastic sealer,
- ELISA reader

**Procedure:**

- The microtitre wells are fitted in the frame provided and labelled.
- Well A1 is marked as blank, B1, C1 marked as negative control and D1, E1, F1 as positive control.
- 100µl of ready to use controls are added to corresponding wells. 100µl of dilution buffer and 10 µl of samples are added.
- The microtitre plate is incubated for 30minutes at room temperature (25-30ºC).
- Wells are washed 5 times with working wash buffer.
- 100 µl of working HRP conjugate is added to each well and incubated for 30minutes at room temperature (25-30ºC).
- Wells are washed 5 times with working wash buffer.
- 100 µl of working TMB substrate is added to each well.
- Plate is incubated for 30minutes at room temperature (25-30ºC).
- 100µl of stop solution is added.
- The absorbance is read at 450/630nm wavelength.

Cut off formula: (0.1×PC) + 0.1

**Validation:**

| Control | Value |
|---|---|
| Blank | < 0.15 |
| Negative control | < 0.25 |
| Positive control | >0.60 |

**Procedure for HCV PCR:**

Principle of real time PCR is based on the amplification of specific regions of the viral genome detection of amplified product via fluorescent dyes. Specific fluorescent dyes linked to oligonucleotide probes which bind to the amplified product. Detection and quantitation of the accumulating product is by monitoring fluorescent signal during PCR run without having to re-open the reaction tubes after the PCR run. First step in real time PCR is reverse transcription step. Primers were designed to amplify a 240 bp fragment within the 5' non-coding region of the HCV genome.

**Requirements:**

- RNA isolation kit
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer

- Benchtop centrifuge
- Bio – Rad thermo cycler
- Strip Tubes and Caps
- Cooling block

**RNA Extraction:**

KIT: Qiagen extraction kit

**Components:**

- QIA amp Mini Spin Columns
- Collection Tubes (2ml)
- AVL lysis buffer
- Molecular grade ethanol
- Aqueous wash buffer 1
- Aqueous wash buffer 2
- Elution buffer (AVE)
- Carrier RNA (poly A)

Internal control is used to monitor the RNA isolation procedure and PCR inhibition.

**Preparation of reagents:**

Carrier RNA: 310$\mu$l of Buffer AVE is added to a vial of carrier RNA

Buffer AVL: 310 µl of prepared RNA mixed to a bottle of Buffer AVL (31ml), stored at 4ºC

Buffer AW1: 125 ml of Ethanol is added to a bottle of 95ml Buffer AW1

Buffer AW2: 160 ml of Ethanol is added to a bottle of 66ml Buffer AW2

**RNA extraction Procedure**

- 560 $\mu$l of Buffer AVL is added into 1.5ml specimen tube.
- 140 µl of the specimen transferred to AVL buffer tube, mixed well, Vortexed and Incubated for 10 minutes at ambient temperature.
- 560 µl of ethyl alcohol is added to the sample.
- 630 µl from mixture is added to the QIAmp spin column and Centrifuged for one minute at 8000rpm.
- Collection tube is removed and spin column is transferred to a new 2 ml tube.
- Remaining 630 µl of the above solution is added and centrifuged for one minute at 8000rpm.
- Collection tube changed and the spin column is fixed in a new tube.
- 500 µl of AW1 is added and centrifuged for 1 minute at 8000rpm.
- Tube containing filtrate is discarded and the spin column is transferred to new tube.

- 500 µl of Buffer AW2 is added and centrifuged for 3 minutes at 14000rpm.
- The spin column is fixed in a 1.5 ml Microfuge tube with cap. 60 µl of Buffer AVE is added and kept in a room temperature for one minute.
- Centrifuged for 8000rpm for 1 minute to elute RNA

**Internal control:**

The internal control is added in the isolation procedure at a ratio of 0.1µl per 1µl elution volume or directly to the mixture of Hep. C Virus RG Master A and Hep. C Virus RG Master B to check PCR inhibition.

**Nucleic acid amplification by real time RT-PCR:**

HCV RNA detection is done by real time RT-PCR, which is based on Taqman principle. During PCR forward and reverse primers hybridize to a specific sequence product. Taqman probe, is contained in the same reaction mixture, which consists of an oligonucleotide labelled with a 5'-reporter dye and a downstream, 3'-quencher dye. It hybridizes and possesses 5'-3' exonuclease activity cleaves the probe. The reporter and quencher dye are separated upon cleavage, resulting in an increase in fluorescence for the reporter. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

**PCR KIT:**

Qiagen's Artus HCV RG RT- PCR Kit

Number of reactions : 24

**Contents:**

- Hep. C Virus RG Master A
- Hep. C Virus RG Master B
- Hep C Virus RG QS 1*($10^4$IU/µl)
- Hep C Virus RG QS 2*($10^3$IU/µl)
- Hep C Virus RG QS 3*($10^2$IU/µl)
- Hep C Virus RG QS 4*($10^1$IU/µl)
- Hep C Virus RG IC
- Water (PCR Grade)

**Storage and stability:**

The components of the Artus HCV RG RT-PCR Kit were stored at –30°C to –15°C.

**Real time RT-PCR procedure:**

Required number of PCR tubes placed in thee adapters of the precooled block that includes tubes for extracted samples, PC and atleast one NC (PCR grade water). All samples are thawed completely and mixed well.

**Preparation of reaction mix/run**

| | |
|---|---|
| Hep. C Virus RG Master A | - 12µl |
| Hep. C Virus RG Master B | - 18 µl |
| Hep C Virus RG IC | - 2 µl |
| Total volume | - 32µl |

**Preparation of PCR assay/run**

| | |
|---|---|
| Master Mix | - 30 µl |
| Sample | - 20 µl |
| Total volume (Reaction volume) | - 50 µl |

- 30 µl of the master mix is added into each PCR tube.
- 20 µl eluted sample RNA is added.
- One of the quantitation standards must be used as positive control
- 20 µl of water is added as a negative control.
- PCR tubes are placed in thermocycler.

**Programming thermocycler (Bio-Rad) for PCR amplification**

| | |
|---|---|
| Reverse transcription | - 50ºC for 30Minutes |
| Taq inhibitor activation | - 95 ºC for 15minutes |

**Cyclic conditions (50 cycles)**

| | |
|---|---|
| Denaturation | - 95 ºC for 30seconds |
| Annealing | - 50 ºC for 60seconds |
| Extension | - 72 ºC for 30seconds |

**PCR amplification steps**:

**Reverse transcription:**

The *RNA* template is converted into cDNA using a reverse transcription.

**Denaturation:**

The first step in the amplification procedure is denaturation. The thermocycler raises the temperature to 95ºC for 30seconds for Taq enzyme activation. The hydrogen bonds holding the complimentary strands of DNA together are broken.

**Annealing:**

When temperature is decreased to 50 ºC for 60 seconds the complementary binding of the two specific oligonucleotide primers to the DNA template take place.

**Extension:**

The DNA polymerase extends the primers when the temperature is increased to 72 ºC for 30 seconds. The template DNA is synthesised using

deoxynucleotides in the mixture. The template plate DNA and newly synthesised complementary DNA strands join together to form new double stranded DNA copies. The newly formed DNA copies acts as a template for further amplification. The reaction product of each cycle was detected using fluorescence signal.

**Interpretation:**

- No HCV RNA is detectable - No signal is detected in fluorescence channel Cycling A. FAM (Green), with positive signal from the internal control in the Cycling Orange channel.
- HCV RNA detectable- A signal is detected in fluorescence channel Cycling A. FAM (Green), ROX (orange) - The result of the analysis is positive.
- Result cannot be concluded when the signal is not detected in both channels

**HCV genotyping by real time RT- PCR:**

**Kit:** Geno Sen' HCV 1/2/3/4 (Rotor Gene) Qualitative Real Time PCR Kit

**Contents:**

- HCV Genotyping Super mix(R1)
- HCV Genotyping Mg sol RT (R2)
- HCV Genotyping Positive control ( only Genotype 1,2,3,4)
- Molecular grade water

**Procedure:**

Required number of PCR tubes placed in the cooling block. All reagents and samples are thawed completely before use and mixed by pipetting.

**HCV Genotyping Master Mix preparation**

HCV Genotyping Super Mix -15µl

HCV Genotyping Mg. Sol - 5 µl

Total volume (Reaction volume) - 20 µl

- 20 µl of Master Mix added into each labelled PCR tube.
- 30 µl extracted sample RNA positive control and Water (PCR} as a negative control is added into the corresponding tubes.
- PCR tubes are closed and transferred to rotor of the Rotor Gene instrument.

**Cyclic condition:**

Reverse Transcription - 50ºC for 15 minutes

Taq inhibitor activation - 95º for 10 minutes

**PCR amplification**

Number of cycles -45

Denaturation - 95ºC for 15 seconds

Annealing - 55ºC for 20 seconds

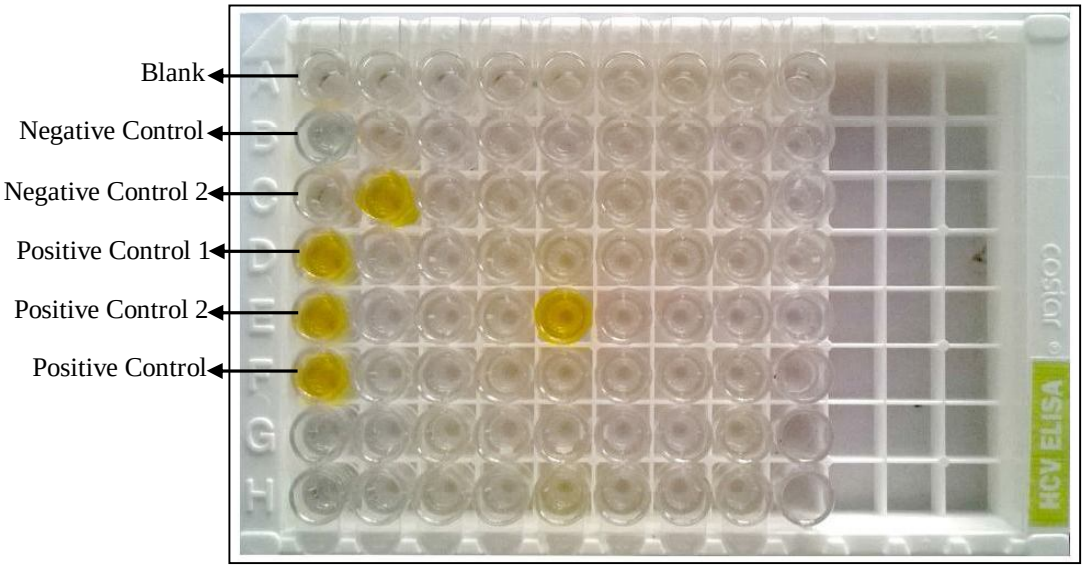
Extension - 72ºC for 15 seconds

**Interpretation:**

- HCV Genotype 1- Signal is detected in fluorescence channel cycling A. Red.
- HCV Genotype 2 - Signal is detected in fluorescence channel cycling A. Green
- HCV Genotype 3- Signal is detected in fluorescence channel cycling A. Orange –
- HCV Genotype 4 - Signal is detected in fluorescence channel cycling A. Yellow –
- If signal is not detected in any of the above fluorescence channel cycling- Genotype other than 1,2,3,4.
- If signal is not detected in fluorescence channel cycling A. Green, Yellow, Orange, and Red for samples and positive control– Troubleshooting. Repeat the assay.

**FIG 1: HEPASCAN HCV ELISA KIT**


**FIG 2: RESULTS - ANTI HCV ELISA TEST**



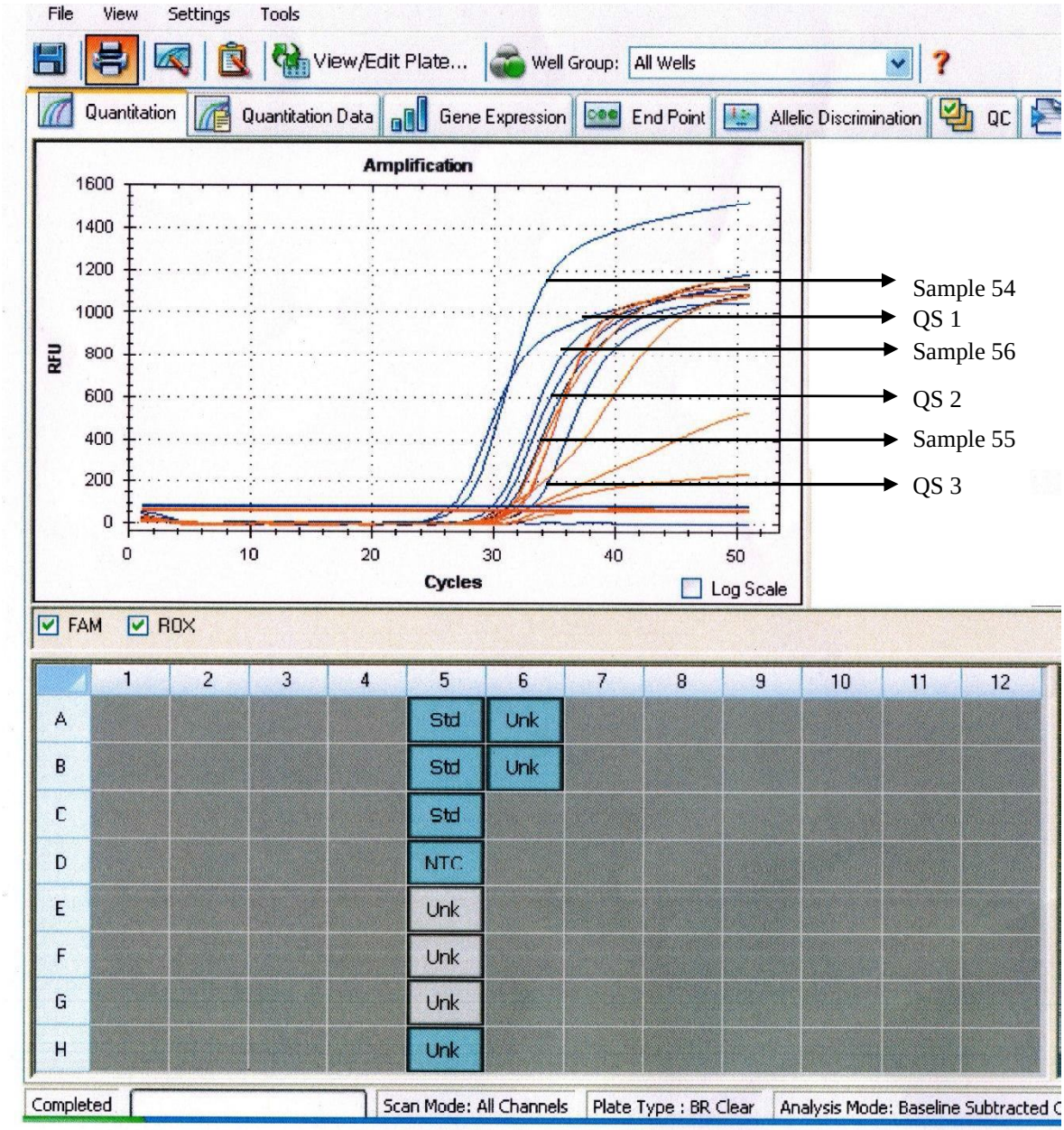
## FIG 3: QIAGEN RNA EXTRACTION KIT


## FIG 4: QIAGEN NUCLEIC ACID EXTRACTOR

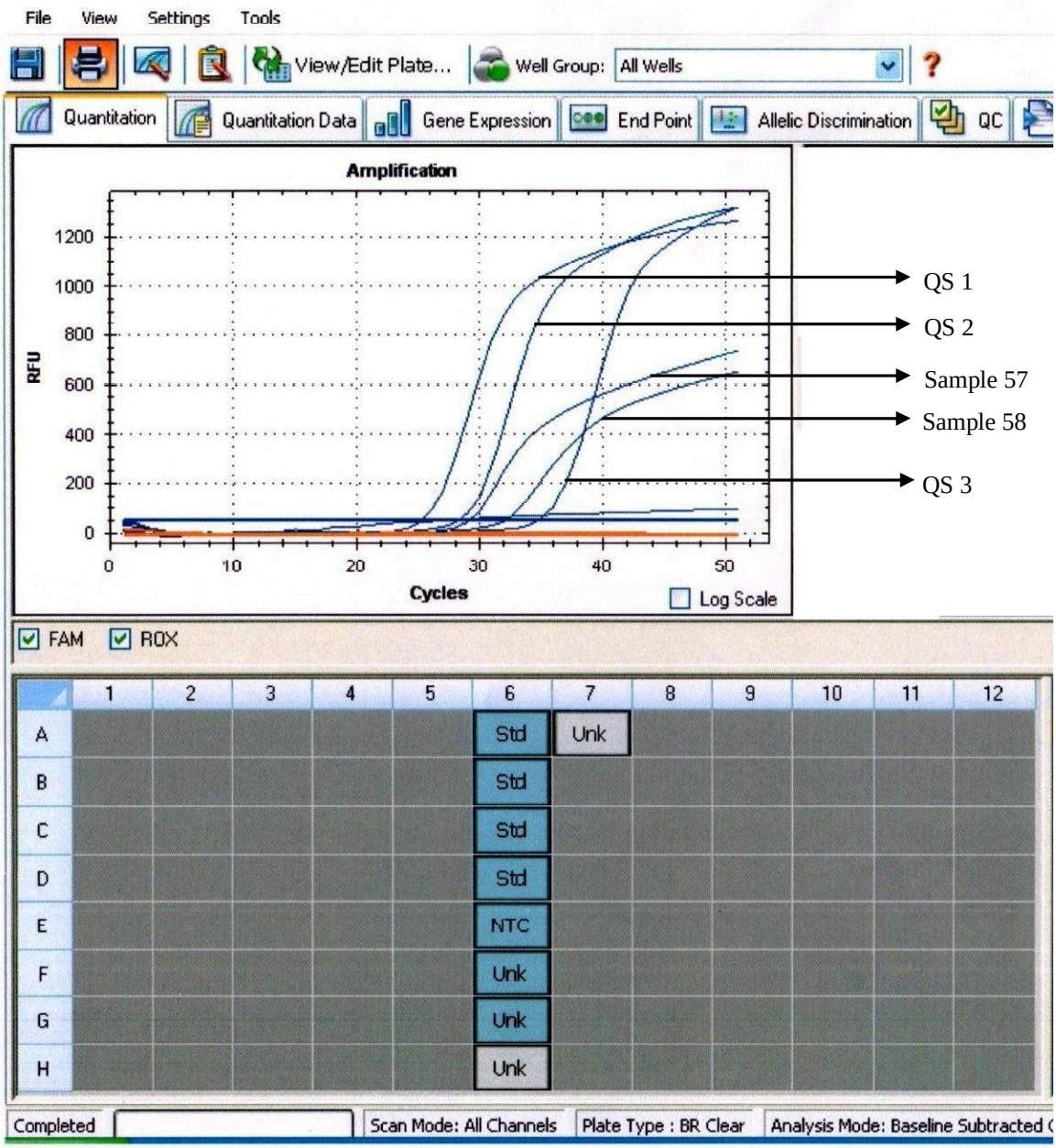
**FIG 5: QIAGEN HCV RNA PCR KIT**


**FIG 6: THERMAL CYCLER – BIO RAD CFX96 REAL TIME SYSTEM**

# FIG 7: HCV RNA QUANTITATION BY RT - PCR - GRAPH

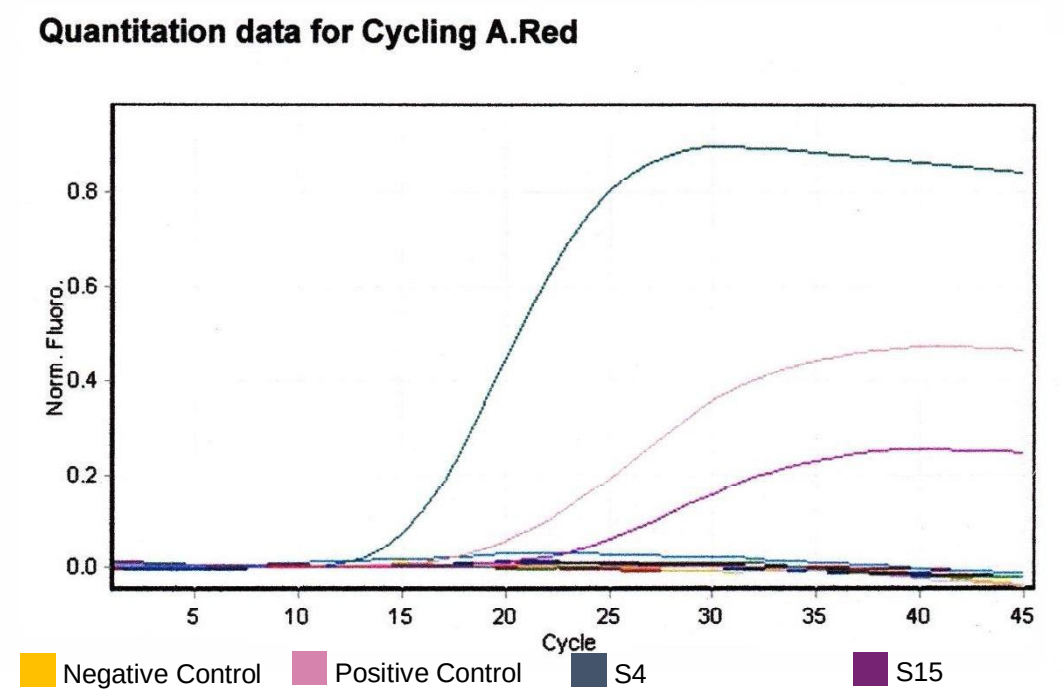


## FIG 8: HCV RNA QUANTITATION BY RT - PCR - GRAPH



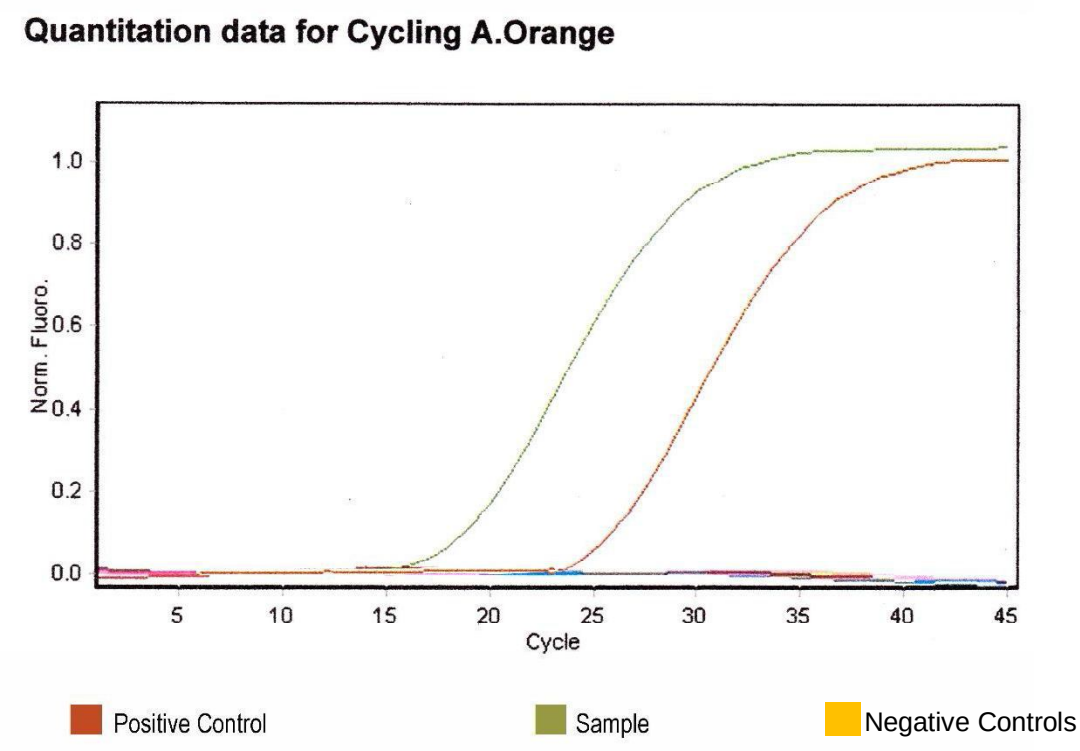
## FIG 9: HCV GENOTYPING BY RT – PCR - GRAPH

## GENOTYPE 1



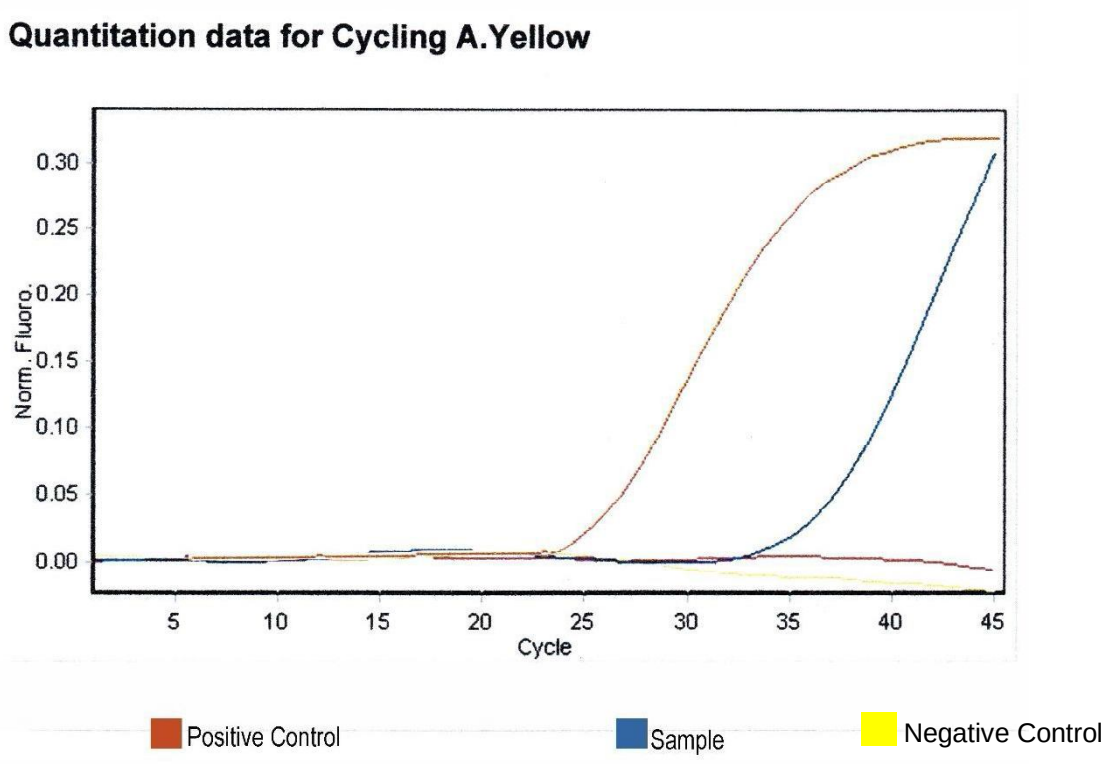

## FIG 10: HCV GENOTYPING BY RT – PCR - GRAPH

## GENOTYPE 3



## FIG 11: HCV GENOTYPING BY RT – PCR - GRAPH

### GENOTYPE 4



# RESULTS

## RESULTS

**Description of study population:**

200 patients with clinical, biochemical and ultrasonographic evidence of chronic liver disease enrolled in the present study. The study group comprised of 61 cases of chronic hepatitis, 133 cases of cirrhosis and 6 cases of hepatocellular carcinoma.

**Age distribution:**

The age-wise distribution of the patients in the study group ranges from 21 to 70 years , with a mean of 45. The majority of the cases belong to age group between 41 to 70 years (76.5%). Age of the patients (Refer table:1)

**Gender distribution**

Among 200 patients under study group, 110(55%) were males and 90(45%) were females respectively (Refer table: 2)

**Serological profile of study population:**

The serological assay was done for detection of Anti-HCV antibodies. Out of 200 patients tested for Anti-HCV antibodies by ELISA, 56(28%) were positive. (Refer table no: 3)

Out of 61 cases of Chronic Hepatitis, 14 (22.9%) and from 133 cases of cirrhosis 38 (28.6%) were positive for Anti- HCV antibodies by ELISA.

**Age specific distribution:**

Age-wise analysis in the present study showed high sero- positivity among individuals in the age group of 31-40 years (31.8%) followed by age group 41-50 years (30.1%) and 51-70 years (27%). Lowest prevalence observed in age group greater than 70 years (15.3%) and 21-30 years (16.67%). (Refer table no 4)

**Gender distribution:**

Among the 56 Anti-HCV positive patients, 33(30.8%) and 23(24.7%) were males and females respectively. (Refer table 5)

**Liver enzyme profile:**

Liver enzymes in HCV related chronic liver disease are may be fluctuating or normal. In the present study showed ALT was elevated in 35.7%, AST in 30.35% and Alkaline phosphatase in 33.9% of Anti-HCV positives.

The present study also showed that serum bilirubin was elevated only in 2.8% of Anti-HCV positives. (Refer table 7)

**Risk factors distribution**:

In the present study, the probable risk factor for HCV transmission was observed as blood transfusion due to non-surgical causes in 11 cases (29.7%), surgery and blood transfusion in 8 cases (19%), haemodialysis in 5 cases (23.8%), unsafe injection in 6 cases (19%) and IV drug abuse in

only one case (33%). No risk factors were identified in other Anti-HCV positive cases (40%). (Refer table 8)

**Molecular assay:**

Out of 200 samples tested for the presence of HCV RNA by real time only RT-PCR, 29 (14.5%) were detected to have HCV RNA. 26(13%) samples had been found to be positive by both ELISA and PCR. Among 144 ELISA negative samples, 3 (2.1%) were PCR positive. Thirty ELISA positive samples were tested negative by PCR. (Refer table 9)

**Genotype distribution:**

All the HCV RNA positive samples were subjected to genotype determination by real time RT-PCR. Genotype 3 was most common type observed in 17(58.6%) in this study followed by the genotype 4 which was seen in 6 (20.6%) cases. Four cases (13.7%) showed genotype 1 and genotype other than 1,2,3,4 was also observed in 2 (6.9%) cases. (Refer table 10)

Genotype 3 is the more prevalent genotype in the age group 31 – 70 years and also in both genders (Refer table 12)

**TABLE: 1**

**AGE-WISE DISTRIBUTION OF STUDY POPULATION**

| Age group(in years) | Number of patients (n=200) | Percentage |
|---|---|---|
| 21-30 | 12 | 6% |
| 31-40 | 22 | 11% |
| 41-50 | 53 | 26.5% |
| 51-60 | 48 | 24% |
| 61-70 | 52 | 26% |
| >70 | 13 | 6.5% |

**TABLE: 2**

**GENDER-WISE DISTRIBUTION OF STUDY POPULATION**

| GENDER | NUMBER OF PATIENTS | PERCENTAGE |
|---|---|---|
| MALE | 110 | 55% |
| FEMALE | 90 | 45% |
| TOTAL | 200 | 100% |

**TABLE NO: 3**

**SERO-PREVALENCE OF HCV AMONG CHRONIC LIVER DISEASE PATIENTS**

| No of chronic liver disease patient | No of Anti-HCV positive cases (by ELISA) | Percentage |
|---|---|---|
| 200 | 56 | 28% |

**TABLE NO: 4**

**AGE- WISE DISTRIBUTION OF ANTI-HCV POSITIVE CASES**

| Age groups | No of cases (n=200) | ELISA positive cases |
|---|---|---|
| 21-30yrs | 12 | 2(16.67) |
| 31-40yrs | 22 | 7(31.8%) |
| 41-50yrs | 53 | 16(30.1%) |
| 51-60yrs | 48 | 13(27%) |
| 61-70yrs | 52 | 14(26.9%) |
| >70yrs | 13 | 2(15.3%) |

**TABLE: 5**

**GENDER DISTRIBUTION OF ANTI- HCV POSITIVE CASES (n=200)**

| Gender | No of cases | Anti- HCV positive cases |
|---|---|---|
| Male | 110 | 33(30%) |
| Female | 90 | 23(25.5%) |

**TABLE: 6**

**DISTRIBUTION OF ANTI-HCV AND PCR POSITIVE CASES BASED ON CLINICAL PRESENTATION**

| Clinical presentation | No of cases (n=200) | ELISA positive Cases | PCR positive Cases |
|---|---|---|---|
| Chronic hepatitis | 61 | 14(22.9%) | 8(13%) |
| Cirrhosis | 133 | 38(28.6%) | 18(13.5%) |
| Hepatocellular carcinoma | 6 | 4(66.7%) | 3(50%) |

**TABLE: 7**

**BIOCHEMICAL PROFILE IN ANTI-HCV POSITIVE CASES (n=56)**

| LIVER ENZYME | Normal | Elevated |
|---|---|---|
| Alanine aminotransferase(ALT) | 36 (64.2%) | 20 (35.7%) |
| Asparate aminotransferase(AST) | 39(69.65%) | 17(30.35%) |
| Serum alkaline phosphatase | 37(66.1%) | 19(33.9%) |
| Serum Bilirubin | 32(57.14%) | 24(42.8%) |

**TABLE: 8**

**DISTRIBUTION BASED ON PROBABLE HISTORY OF EXPOSURE TO HCV INFECTION**

| RISK FACTOR | No of cases (n=200) | No of Anti-HCV Positive cases | NO of HCV RNA Positive cases |
|---|---|---|---|
| Blood transfusion due to non-surgical cause | 37 | 11 (29.7%) | 4(10.8%) |
| Haemodialysis | 21 | 5(23.8%) | 4(19%) |
| Surgery and Blood transfusion | 42 | 8(19%) | 5(12%) |
| Unsafe injection | 32 | 6(19%) | 1(3.1%) |
| IV drug abuse | 3 | 1(33%) | - |
| Unknown | 65 | 26(40%) | 15(23.1%) |

**TABLE NO: 9**

**COMPARISION OF RESULTS: ELISA AND PCR (n=200)**

| Result | ELISA +ve Cases | | PCR +ve cases | | Both +ve cases | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| Positive | 56 | 28% | 29 | 14.5% | 26 | 13% |
| Negative | 144 | 72% | 171 | 85.5% | 141 | 70.5% |

**TABLE: 10**

**PREVALENCE OF HCV GENOTYPES AMONG CHRONIC LIVER DISEASE PATIENTS**

| Genotype | Genotype 1 | Genotype 2 | Genotype 3 | Genotype 4 | Genotype other than 1,2,3,4 |
|---|---|---|---|---|---|
| No of cases(n=29) | 4(13.7%) | 0(0%) | 17(58.6%) | 6(20.6%) | 2(6.9%) |

**TABLE: 11**

**PREVALENCE OF HCV GENOTYPES**

**AMONG VARIOUS AGE GROUPS**

| Age groups | Genotype 1 | Genotype 3 | Genotype 4 | Genotype other than 1,2,3,4 | Total |
|---|---|---|---|---|---|
| 21-30yrs | 0 | 0 | 2 | 0 | 2 |
| 31-40yrs | 0 | 3 | 1 | 0 | 3 |
| 41-50yrs | 1 | 5 | 3 | 0 | 9 |
| 51-60yrs | 1 | 2 | 0 | 1 | 4 |
| 61-70yrs | 2 | 7 | 0 | 1 | 10 |
| Total | 4 | 17 | 6 | 2 | - |

**TABLE: 12**

**GENDER BASED DISTRIBUTION OF HCV GENOTYPES**

| Genotype | Male | Female |
|---|---|---|
| Genotype 1 | 1(25%) | 3(75%) |
| Genotype 2 | - | - |
| Genotype 3 | 10(58.8%) | 7(41.2%) |
| Genotype 4 | 5(83%) | 1(16%) |
| Genotype Other than 1,2,3,4 | 1(50%) | 1(50%) |

**CHART 1**

**AGE-WISE DISTRIBUTION OF STUDY POPULATION**

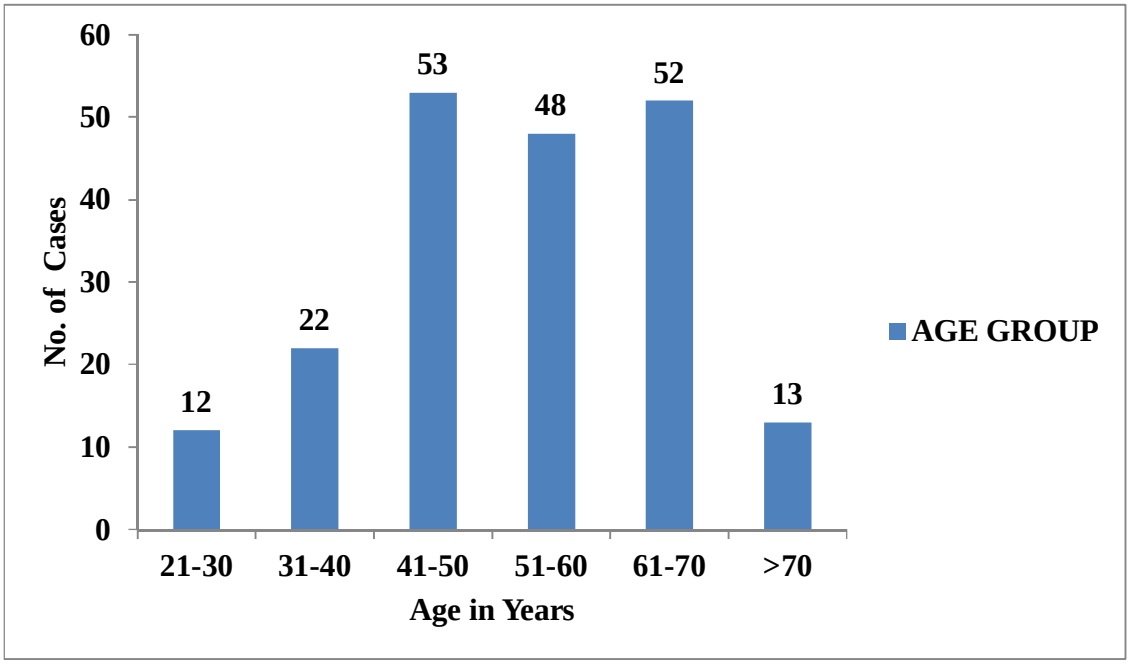
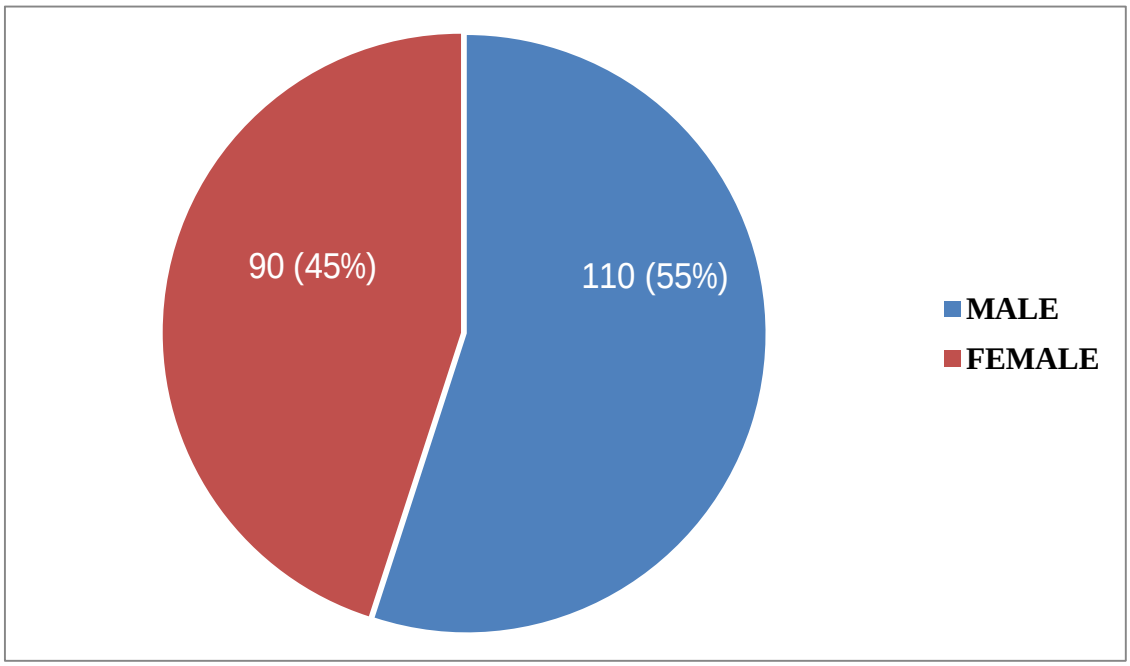


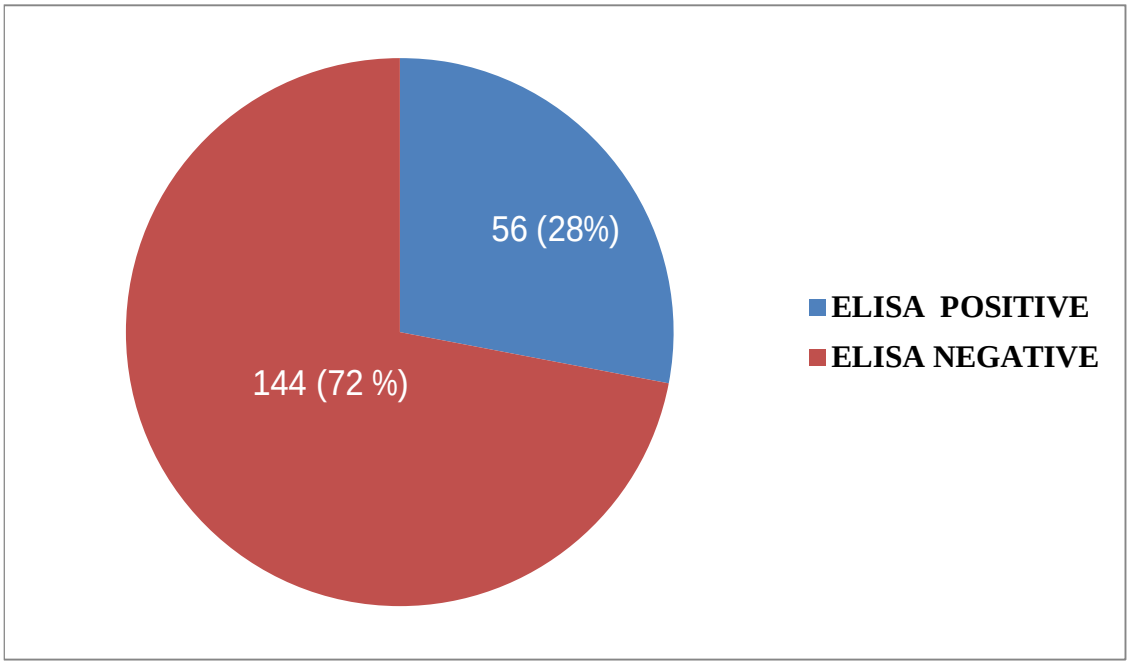

**CHART 2**

**GENDER-WISE DISTRIBUTION OF STUDY POPULATION**



## CHART 3

## SERO-PREVALENCE OF HCV AMONG CHRONIC LIVER DISEASE PATIENTS




## CHART 4

## AGE- WISE DISTRIBUTION OF ANTI-HCV POSITIVE CASES

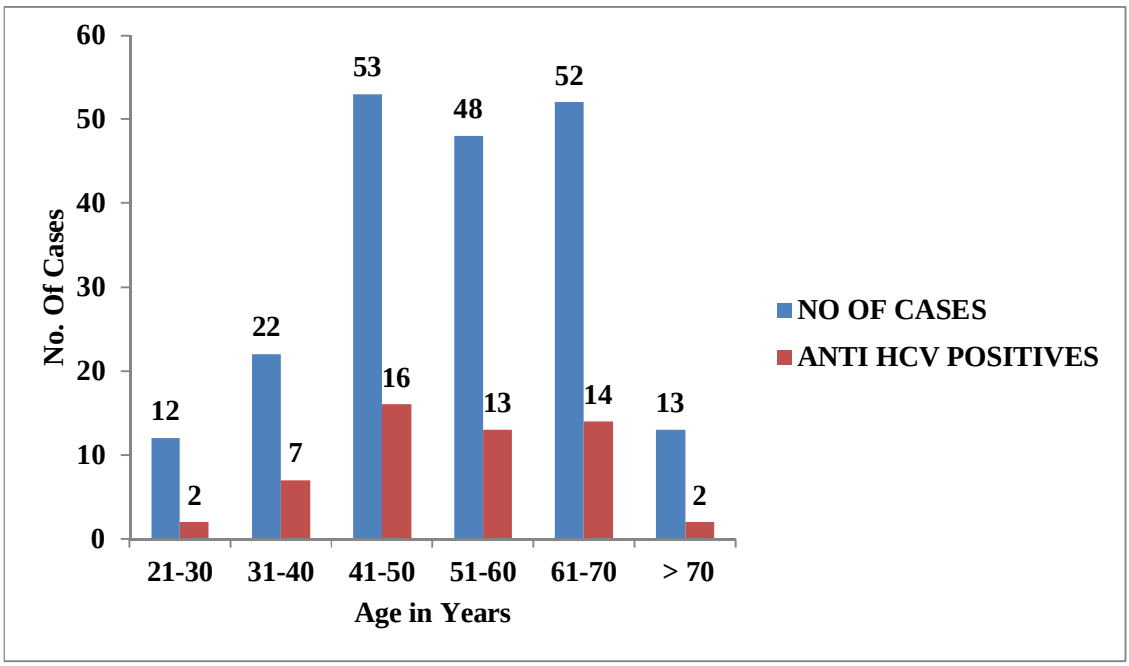


**CHART 5**

**GENDER DISTRIBUTION OF ANTI- HCV POSITIVE CASES (n=200)**

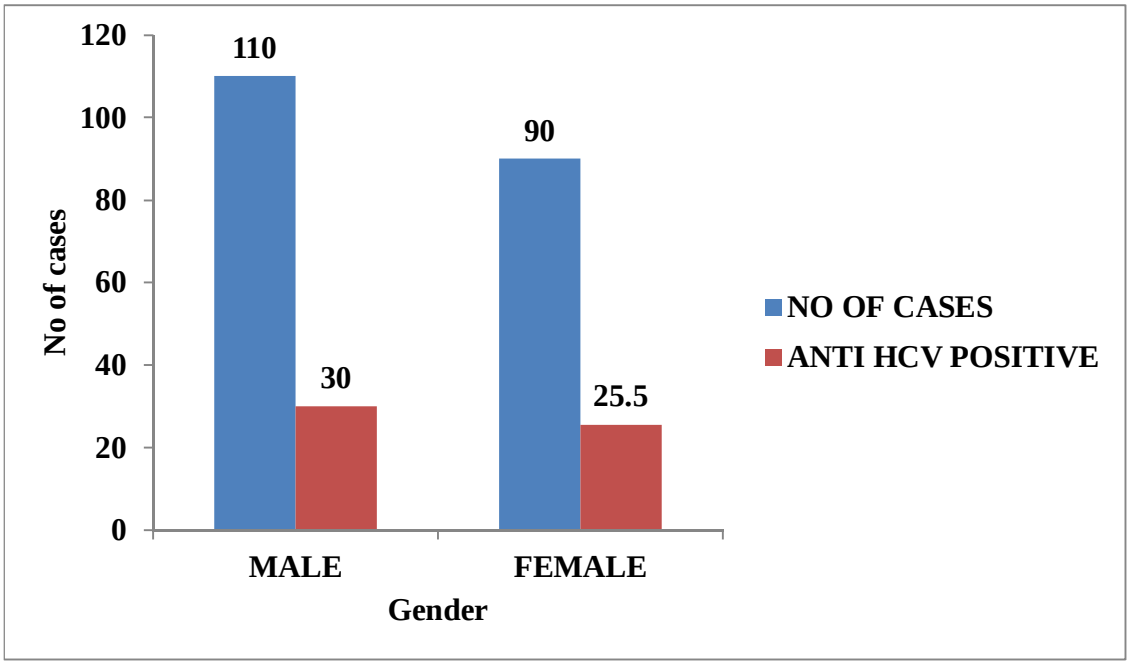
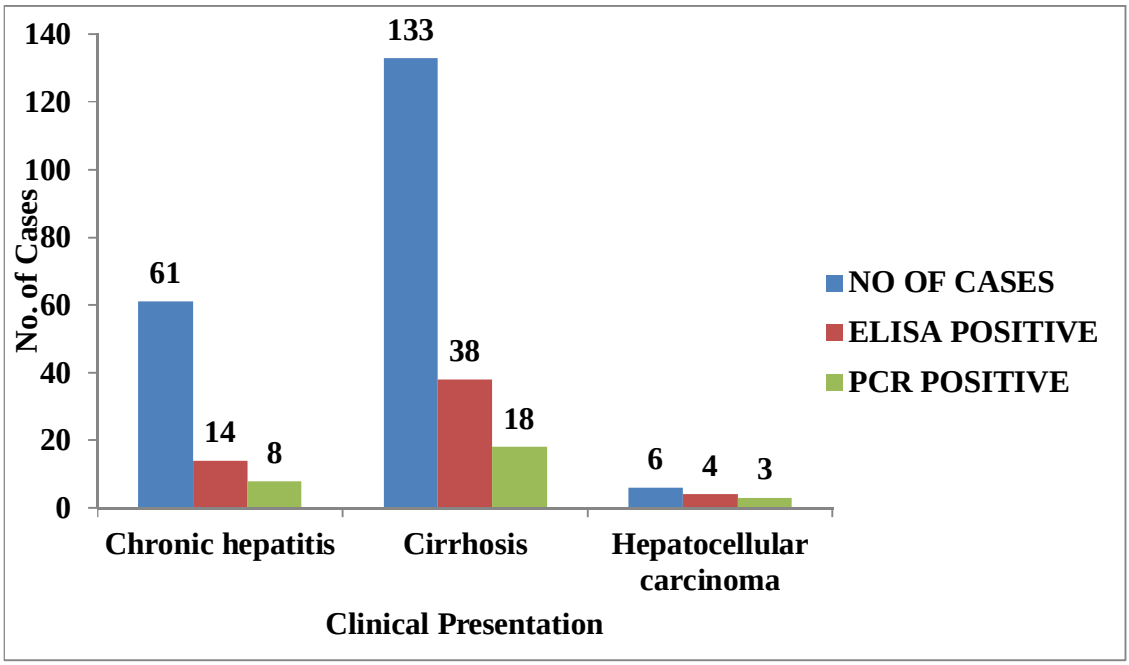


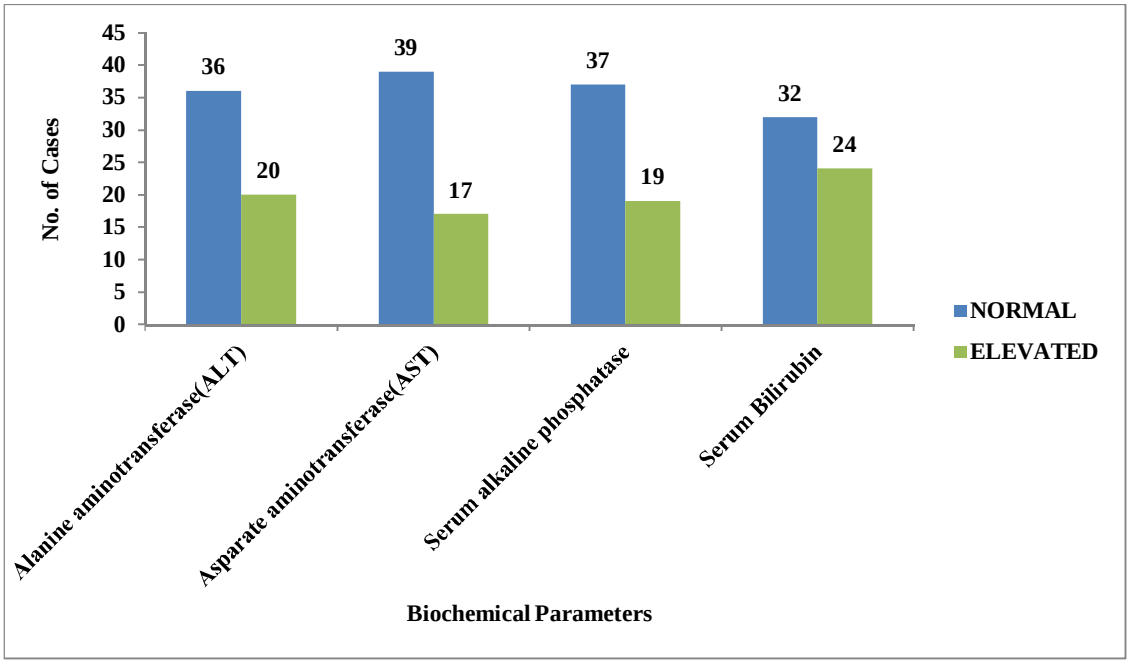

**CHART 6**

**DISTRIBUTION OF ANTI-HCV AND PCR POSITIVE CASES BASED ON CLINICAL PRESENTATION**



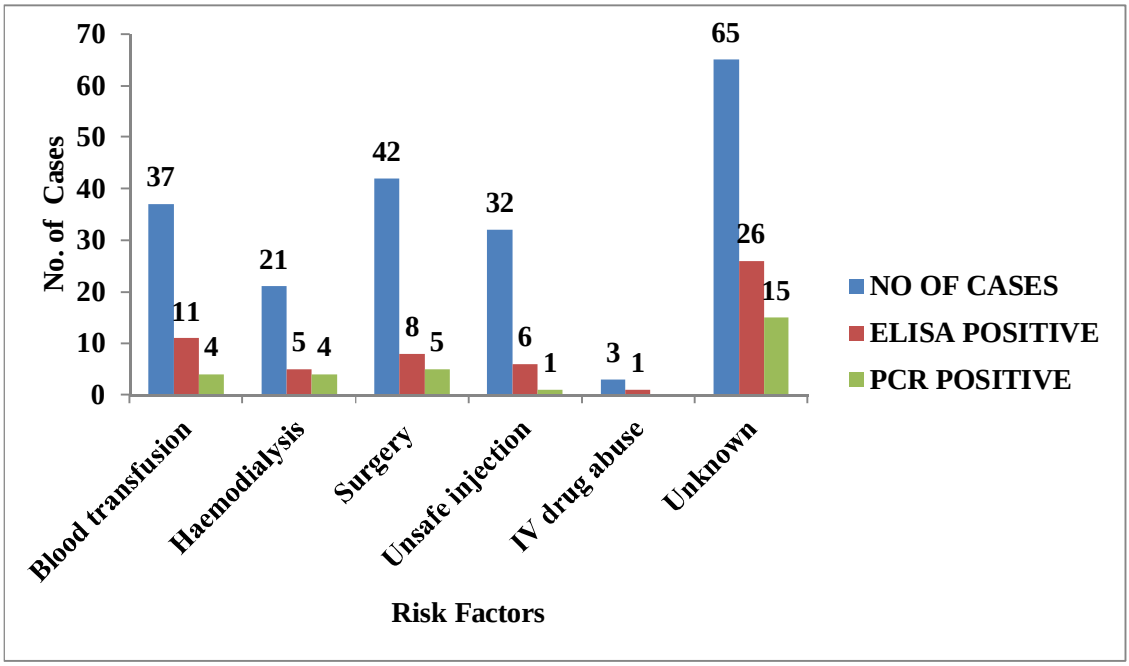
## CHART 7

## BIOCHEMICAL PROFILE IN ANTI-HCV POSITIVE CASES (n=56)



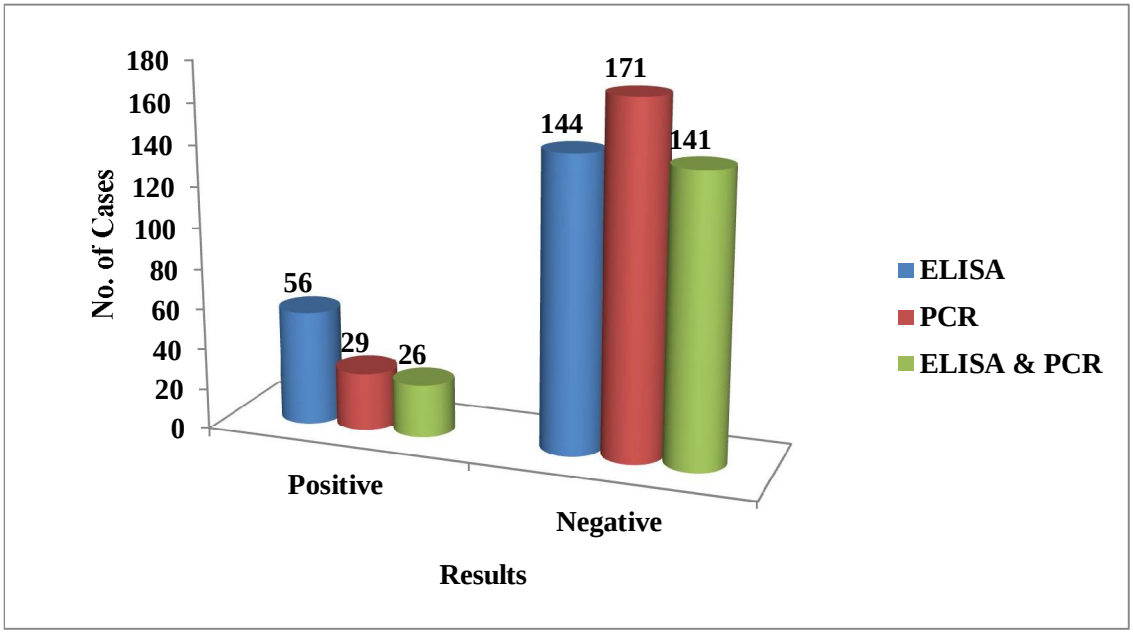

## CHART 8

## DISTRIBUTION BASED ON PROBABLE HISTORY OF EXPOSURE TO HCV INFECTION



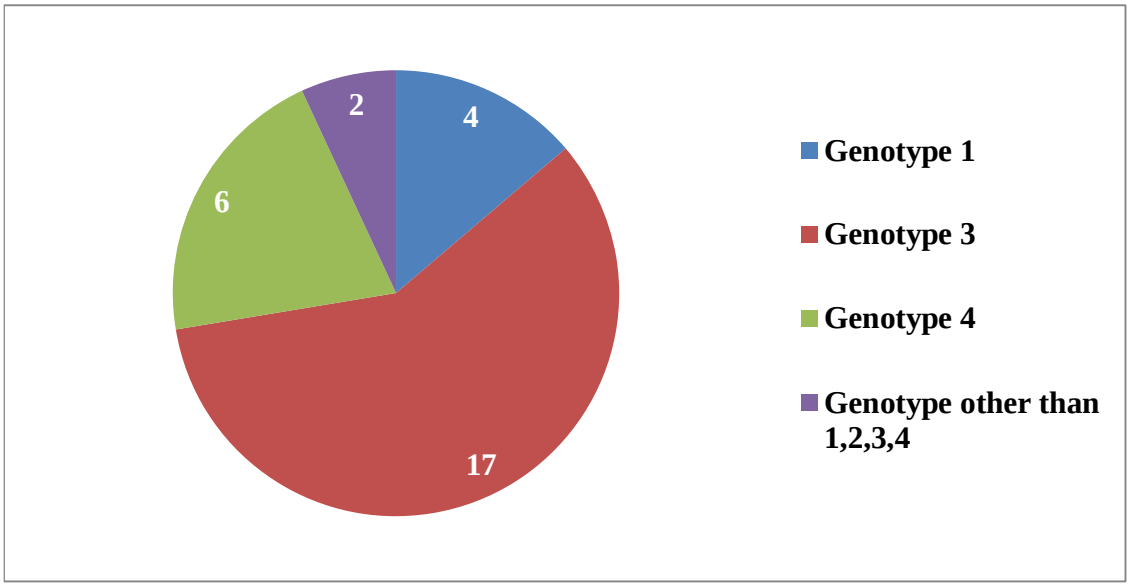
## CHART 9

## COMPARISION OF RESULTS: ANTI-HCV ELISA AND HCV RNA PCR (n=200)



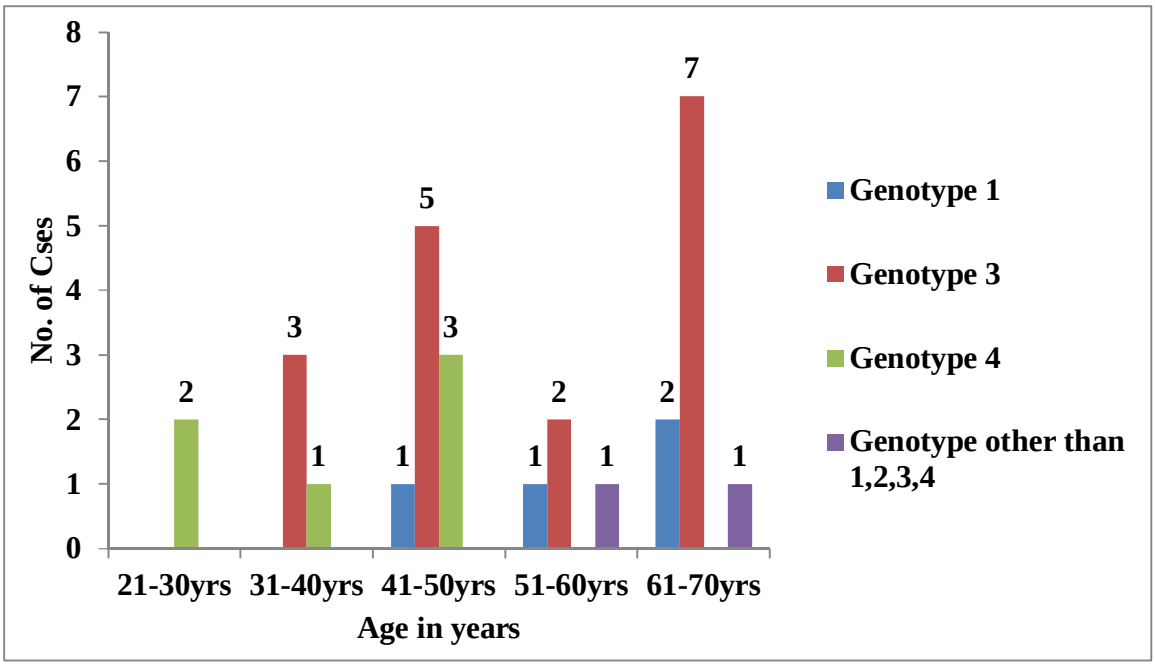

## CHART 10

## PREVALENCE OF HCV GENOTYPES AMONG CHRONIC LIVER DISEASE PATIENTS



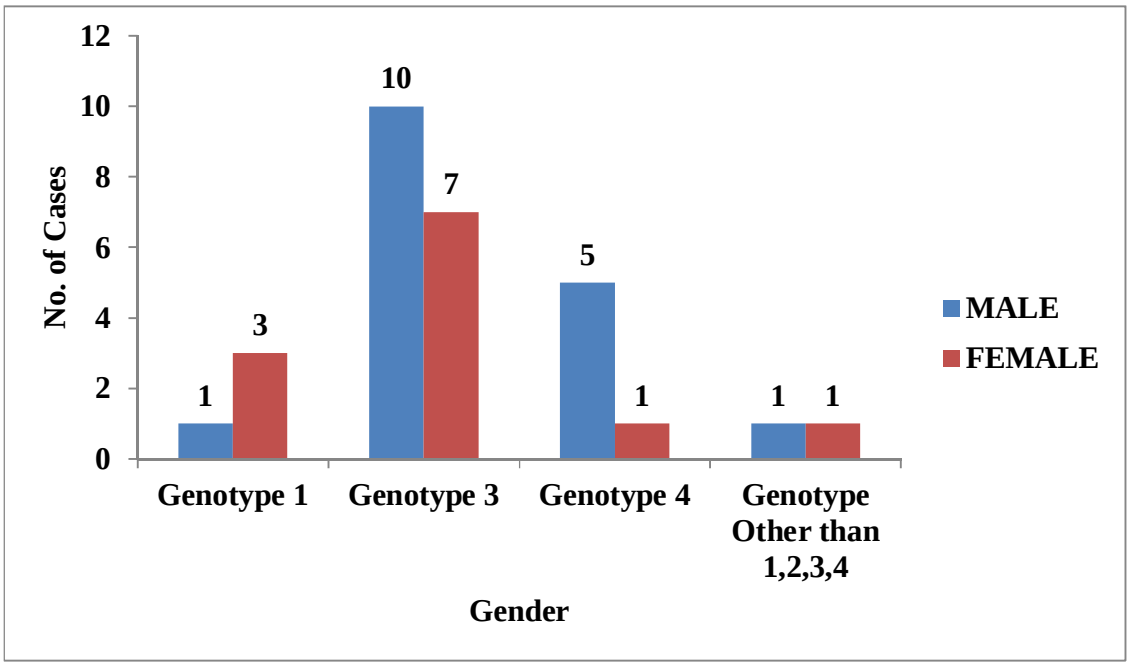
## CHART 11

## PREVALENCE OF HCV GENOTYPES AMONG VARIOUS AGE GROUPS




## CHART 12

## GENDER BASED DISTRIBUTION OF HCV GENOTYPES AMONG CHRONIC LIVER DISEASE PATIENTS



# DISCUSSION

## DISCUSSION

Hepatitis C virus infection is a serious threat to health care system. It can cause varying clinical conditions ranging from acute infection to chronic hepatitis and hepatocellular carcinoma. The sero-prevalence of HCV varies in different parts of the country. The complex and uncertain nature of HCV infection and its chronicity emphasises the difficulties in prevention and control of HCV.

The information about sero-prevalence of HCV among chronic liver disease cases and relative distribution of each genotype are essential tool to formulate effective treatment strategies.

In this study, 200 chronic liver disease cases were included as study population. Patient selection was based on clinical, biochemical and ultrasonographic evidence of chronic liver disease. The patients were subjected to serological test for detecting antibodies. Detection of HCV RNA & genotyping is done by molecular assay.

In the present study, 200 CLD patients were screened and 56 (28%) were tested positive for Anti-HCV antibodies. The prevalence among chronic liver disease patients has been high when compared with general population (0.87%)[17]. This sero-prevalence rate is comparable with the sero-prevalence of Anti-HCV 18% by Anirban Kundu et al (2013) and 22.5% by Abel Girma Ayele et al (2015), and 26% Issar SK et al (1995) and other

studies reported previously[50,51,52,53]. This implies that there has been no appreciable change in the sero-prevalence of HCV over the last 10 year period. Much lower sero-prevalence of 4% was observed by Sanjay Sharma et al (2006), 5.7% by A Blankson et al (2005), and 8.2% observed by Ganesh Kumar Anbazhagan et al (2010)[57, 60, 61,]. In relation to variation of HCV sero-prevalence, the reasons cannot be completely discerned. However, the difference in demographic characteristics of the study population, the difference in hepatitis epidemiology, awareness of the routes HCV transmission, efforts made to implement universal precautions by health professionals and preliminary benefits due to the mandatory HCV screening while blood donation and prior to any surgical procedure might explain these discrepancies.

The majority of the CLD cases belong to age group 31-70 (76.5 %). Sero-prevalence was highest among persons in the age group 31-50 years (30.6%) and a substantial prevalence till 70 years of age. This finding goes in correlation with study done by Sandhu et al (2015)[71] and Ganesh Kumar Anbazhagan et al (2010)[61]. The lowest prevalence was observed in the age group of less than 20 years (16.67%) and more than 70 years (15.3%) which is comparable to the other studies by Soin et al (2015)[57], Paramdeep Singh et al (2014)[74]. This sero-prevalence pattern of high age distribution i.e., after 3rd decade of life indicates that the HCV transmission would have either

occurred in their early childhood or during the adulthood period . The chronic HCV infection may lead to progression of liver diseases. Now, it may end up with Cirrhosis, CLD & HCC over a period of 20- 40 years. Regarding gender of Hepatitis C positive patients, this study has shown that there were 33 (30.8%) males and 23 (24.7%) females showing predominance of male gender. Similar findings were reported by Atreyi Chakraborty et al (2015) study[58]. It showed significantly higher prevalence in male (71.6%) patients in comparison with the females (28.4%). Anirban Kundu et al (2015) study showed 18.92% of males were anti HCV antibody positive and 15.39% of females were anti HCV Ab positive[48].

Soin.D.et al, Paramdeep Singh et al, and Ganesh Kumar Anbazhagan et al also have shown male predominance in their studies, which indicates a reasonably higher proportion in males than females in HCV infection could be a reflection of more males coming for treatment in our setting[57,56,16]. Besides it could be due to more social mobility in males than females and thus greater vulnerability to be infected. Another factor may be that in the study group the males outnumbered the females, so there might have been overrepresentation of the males.

Abel Girma Ayele et al (2013), Muhammad Usman Anjum et al (2015), Muhammad Ali Tahit et al (2015) and Charles E Ramarokoto (2008)[51,72,75,76] studies showed female predominance.

The present study showed sero-prevalence was more in rural area than urban. This finding goes in correlation with studies done by Anirban Kundu et al, A Muhammed Ali Tahiret al (2015) and Anuj Sharma et al (2014)[50,76,77]. This could be explained to be due to unsafe injection practices even now prevailing in the rural areas of our country, the most common mode of transmission of blood borne infections like HCV. It needs to be mentioned that the rural population are still dependent upon the untrained paramedics for their treatment needs.

Qu JB et al (2000), Schinichiro et al (1997) studies showed no substantial difference between urban and rural population[79, 80]. High prevalence in urban population observed in Shahin Merat et al (2010), Abel Girma Ayele et al (2013) [81,51] may be due to migration of people from different geographical areas , dense population, change in life style and exposure to various risk factors especially like intravenous drug abuse, multiple blood transfusion and unprotected sexual promiscuity etc.,

Hepatitis C virus is transmitted primarily through the parenteral route and source of infection include transfusion of blood and its products, unsafe therapeutic interventions, drug abuse, needle stick injuries, hairdressing and tattooing.

Most frequent risk factor for HCV transmission in this study was IV drug abuse observed in 33% of cases followed by blood transfusion in 24%, haemodialysis in 22%, surgery in 21% and unsafe injection in 19%.

The prevalence of Anti-HCV antibodies was 33% among intravenous drug users in this study. This finding is also supported by Basu D et al (2013)[66] showed prevalence of 31.8% and study done at YRGCARE Chennai which reported prevalence of 55%. This could be explained by the fact that Injection drug users are at risk for blood-borne pathogen, including Hepatitis C virus, Hepatitis B virus and HIV. In developed countries, HCV is predominantly transmitted by intravenous drug abusers. Drug sharing and preparation practices are responsible for transmission of HCV. Sharing partners are necessary to sustain transmission of HCV virus.

Blood transfusion history was elicited in 24% sero-positive cases. This finding correlates well with study by Amarapurkar et al (2001)[68] which showed 38% and Abel Girma Ayele et al (2013)[49] study with 21.5%. Blood transfusion was responsible for about sixty one percent of cases with chronic HCV infection according to a study from Vellore by Seeff et al (1992)[78]. This could be attributed to the fact that blood transfusion allows a large quantum of infective virions into the susceptible patient.

In the present study, 22% (5/21) of patients with chronic renal failure on long term haemodialysis were found to be positive for anti HCV antibody.

Similar finding was observed by Divya Soin et al (2015) and Rubina Malhotra et al (2016)[57,69]. Multiple blood transfusions and haemodialysis were also significant risk factors observed in this study, which could be explained by the fact that patients on haemodialysis are at an increased risk for acquiring HCV as a result of multiple blood transfusions and cross contamination from dialysis circuit. Stringent blood screening and Strict infection practices in dialysis unit are required for reduction of transmission.

The antibody protection is poor in patients on chronic haemodialysis and after renal transplantation due to immunosuppression. The ELISA alone may fail to detect the HCV infection in these cases. HCV RNA testing should be made mandatory for these patients.

Among kidney transplants, the prevalence was reported to be as high as 55.9% by Radhakrishnan et al (2000) and 26.2% by Gosavi et al (1997), since most of these kidney transplant patients underwent dialysis and received multiple blood transfusion prior to transplant[76,77].

Health care workers have an increased risk of acquiring hepatitis infection. One patient in this study was a staff worked in a dialysis unit got infected and now under antiviral treatment.

In many developing countries like India, an important risk factor for HCV is contaminated syringes. In many places of India, supplies of sterile syringes may not be available or non-medical professionals often give

injection outside the hospital. In this scenario, people may receive multiple contaminated injections over a period of time which increases the risk of HCV infection.

Nineteen percent of cases gave history of unsafe injection in this study. The reason probably could be many patients believe that injectable and intravenous fluids act faster and relieve the symptoms more quickly than oral drugs. A high frequency of injection use, most of which are administered under unsterile conditions, put the patients on risk of acquiring HCV infection.

Qualitative HCV RNA tests are used to confirm the presence of Hepatitis C virus. RT-PCR can be used for quantitative measurement of viraemia for diagnosis in acute HCV infection, in case of anti-HCV-negative chronic hepatitis C carriers, for evaluation of HCV viraemia in asymptomatic patients with normal liver enzymes, for assessing as well as predicting treatment response and also assessing the severity of disease.

Among 200 cases of chronic liver disease tested for HCV -RNA, 29 (14.5%) were positive. This is consistent with the studies by Anita Chakravarti et al (2011) [26], Mohan KV et al (1999)[66]. HCV- RNA detection rate of 24.66% was observed by D. B. Senevirathna et al (2014), 27.95% by

B.Kazemi et al ( 2004)[70], 66.6% by Caldwell SH et al[69], and 73.6% by L.K. Silva et al (2006)[68] .

The variation in the HCV RNA detection rate among sero-positive cases could be due to intermittent viremia or spontaneous resolution of infection. Other possibilities are underestimation of viremia in patients is due to the fact that virus is present only in hepatocytes. Out of 56 cases positive by ELISA , only 26 (46.42%) were confirmed by real time RT-PCR indicating the HCV infection is active.

Sero-positivity among PCR negative patients may be due to cross reaction with non-specific antibody. Hence, all the anti- HCV positive results should be confirmed by testing for HCV RNA.

Alternatively, true antibody-positive participants did not have positive RT-PCR results because they had intermittent viremia or spontaneously cleared HCV infection.

In the present study, 3 samples from patients undergoing dialysis were negative by ELISA, whereas positive by PCR. This may be due to antibody synthesis is low in immunocompromised state of patients like in chronic kidney disease, when compared to normal individual.

The sensitivity and the specificity of ELISA in this study were 89% and 72% respectively. Though it was good enough as a diagnostic assay,

all the ELISA positive cases should be confirmed by testing for HCV RNA, as the specificity of RT-PCR was absolute at high sensitivity (100%), which is not only suitable for clinical diagnosis and also recommended for the HCV screening in order to prevent the transmission of this disease.

This study showed mean of liver enzymes ALT, ALP of 60, 200.52IU respectively. This is accordance with the fact that liver parameters in HCV are fluctuating and sometimes normal. This finding is comparable to study by Usha Arora et al (2007) predominant sign among 2 HCV Ab positive cases.

It is reported in the literature that patients with HCV infection may present in anicteric, icteric or fulminant form. So it can't be distinguished solely by clinical features or by biochemical markers and therefore serology is must.

No case of co-infection like HBV, HIV with HCV was reported in the present study.

HCV positivity in HIV was found to have abnormal liver parameters compared to patients who are not infected with HCV.

Thiagarajan SP et al reported HBV and HCV co- infection has been reported in 12% of patients with chronic liver disease and 11.7%

Hepatocellular carcinoma .HCV superinfection can cause a much more severe liver disease in patients with chronic HBV infection.

Analysis of the HCV genome has shown extreme variability in both structural and non-structural coding regions. This analysis has identified at least six different genotypes which are divided into several subtypes. HCV genotype is the strongest predictor of response to treatment. Different genotype will respond in a different way to alpha interferon.

The distribution of HCV genotypes vary according to geographical region. Genotypes 1, 2 and 3 are widely distributed throughout the world, but the other genotypes are common in particular geographic region. Study of HCV genotypes within a population is a useful for the study of the evolution of HCV infection in different regions.

The present study showed genotype 3 (57.6%) was the major genotype which is similar to studies by Anita Chakravarti et al (2011), Chistdas et al (2013). This is in contrast to findings by Chandra et al (2003), Valliammal et al (1995), Saravanan et al (2008) in which genotype 1 is prevalent. Genotype 1 is observed in 4(13.7%) cases in this study.

The presumption of an Asian origin of HCV genotype 3 depends on a large number of subtypes of HCV genotype 3 that were isolated in this area. This suggests that genotype has been present in Asian population for many centuries.

Genotype 4 is found in 6(23%) patients in our study. This goes similar to findings by Raghuraman.S et al (2004) study. Genotype 4 is mainly seen in Egypt and Middle East, now shows a rise in trend in South India.

Genotype other than 1,2,3,4 is seen in 2 (7.6%) in this study. Genotype 6 is being increasingly reported in India and appears to be somewhat geographically restricted in its distribution. Genotype 5 is confined to South Africa and Central parts of France and has not yet been reported from this region. Genotype 2 was not detected in our study.

As per Gower et al., genotype 1 was the predominant genotype (46%) worldwide followed by genotype 3 (22%), genotype 2(13%) and genotype 4 in 13%.

Genotype 3 was common among older age group 60-70 years whereas genotype 4 was common among 20-50 years. Therefore we can infer that infection due to genotype 3 in this region occurred much earlier in the past compared to other genotypes.

# *SUMMARY*

# SUMMARY

- The study population comprised of 200 patients with clinically diagnosed chronic liver disease, majority belonging to the age group of 41-70 years.
- The present study showed that the sero-positivity by ELISA among clinically diagnosed chronic liver patients was 28% which indicates present or past infection. In the present study, 13% patients were both PCR and ELISA positive indicating active phase of infection.
- Among sero-positive patients 53.6% were PCR negative. ELISA will remain positive for varying period in persons who have cleared infection. False positive ELISA may occur due to non-specific binding or cross reactivity with other infection. Patients with ELISA positivity should be tested for HCV RNA which is an indicator of ongoing infection.
- HCV RNA was found in 5.4% of ELISA negative patients having CRF on long term dialysis due to poor antibody response to Hepatitis C virus infection in these patients.
- Sero-positivity rate was more in the age group of 40-70 years. This may be due long asymptomatic period of infection with symptoms appearing after a long latent period.

- The male population testing positive for Anti-HCV antibody was higher than female population. This may be explained from the fact that males are more prone to harbour the risk factors for this infection like drug abuse and unprotected sex. Several studies have shown that there was increase in fibrosis progression in the male gender.
- Sero-prevalence was more in rural area when compared to urban population duo to lack of health care facility and unsafe injection practices by quacks prevailing in the rural area.
- This study shows that blood transfusion and surgery were the major route of transmission. Serological screening of blood donor population fails to detect acute infection.
- Drug sharing and preparation practices are responsible for transmission in intra venous drug abusers.
- Liver enzymes were moderately elevated in the present study ALT is elevated in 35.7% of cases; Elevated AST was seen in 30.35% and alkaline phosphatase 33.9% of cases. Serum bilirubin level was elevated in 33.9% of patients. Liver parameters were only moderately elevated or normal in chronic HCV patients.
- The present study describes the frequency of genotypes in different age groups & genders which may help in refinement of HCV prevention and therapeutic programs.

- Genotype 3 was the commonest type observed in 58.6% patients which shows good response to therapy
- The genotype 4 was seen in 20.6% patients showing rising trend and associated with poor prognosis.
- Genotype 1 was seen in 13.7% which respond poorly to interferon Alpha. Genotype other than 1,2,3,4 was observed in 2 (6.9%) cases.
- Genotype 3 was a predominant genotype among both genders and in allage groups

# *CONCLUSION*

## CONCLUSION

Hepatitis C is a serious threat with long term complication. Disease burden is likely to increase in the few decades. HCV infection is endemic in many geographical regions.

Sero-prevalence rate was high among CLD patients than general population. Significant association of Hepatitis C virus and chronic liver disease was observed in the present study. In the absence of vaccine, primary prevention of Hepatitis C should be targeted to reduce transmission of virus. High risk people should be provided with education, counselling and screening.

Proper preventive measures like blood screening, proper sterilisation of instruments, and proper disposal of biomedical waste must be stressed.

Third generation ELISA is an useful cost effective screening test for serological diagnosis of HCV. It has many advantages like they are cost effective & easy to use. RT-PCR is recommended in a addition to screening by ELISA to confirm sero-positivity as ELISA may produce false positive results due to non-specific binding antibodies, cross reaction with circulating organisms and in spontaneously cleared cases.

HCV RT-PCR is a highly sensitive and specific method for detecting active infection. Detection of HCV RNA usually precedes the antibody

reactivity in serum. It helps to rule out false negative and weakly positive ELISA with clinical signs and symptoms of HCV.

The present study highlighted that HCV genotype 3 is the predominant genotype among chronic liver disease in our geographical area. It is expected that distribution of other genotype may be due migration of people, changes in high risk behaviour and lifestyle. Knowledge of distribution of genotypes helps in predicting therapeutic response and the duration of treatment.

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# *ANNEXURES*

# CONSENT FORM

You, Shri./ Smt./ Kum. _____, aged ____ years, S/o / D/o / W/o _____, residing at _____ _____ are request to permit you to be a participant in the research study titled "*Study on Sero-prevalence and Genotypes of Hepatitis C Virus in Chronic Liver Disease Patients attending a Tertiary Care Hospital"* conducted by Dr. Theeba V. M., one of the post graduate trainees in the Dept. of Microbiology, Govt. Coimbatore Medical College and Hospital, Coimbatore. You are eligible for the study as per the inclusion criteria. You can ask him any question or seek from him any clarifications about the study which you may have before agreeing to participate in the study.

Name :

Place :

Date :

Signature :

# ஒப்புதல் படிவம்

நோயாளியின் பெயர்:

பாலினம் : வயது:

பெற்றோர் பெயர் :

முகவரி :

அரசு கோவை மருத்துவக் கல்லூரியில் நுண்ணுயிரியல் துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவி மரு. வெ.ம.தீபா அவர்கள் மேற்கொள்ளும் கல்லீரலை தாக்கும் கல்லீரல் சி வைரஸ் பரிசோதனை பற்றிய ஆய்வில் செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு எனது சந்தேகங்களை தெரிவுபடுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

இந்த ஆய்வில் நான் முழு சம்மதத்துடனும், சுயசிந்தனையுடனும் கலந்து கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் என்னைப் பற்றிய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதை தெரிவித்துக்கொள்கிறேன். எந்த நேரத்திலும் இந்த ஆய்விலிருந்து நான் விலகிக் கொள்ள எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இடம் :

தேதி : கையொப்பம் / ரேகை

# HCV-case details

Name of the patient; Age/sex; IP/OP No.; Date:

Address; Contact No:

Present History;

History suggestive of Jaundice:

H/O Hematemesis /melena:

H/O Abdominal distension/Pedal edema:

H/O fever/anorexia/Malaise

Relevant past history;

H/O Blood and blood products transfusion/surgery/unsafe injections and procedures

H/O High risk behavior:

G/E: Anemia/Pedal edema/dyspnea/Jaundice/varicose veins

S/E: CVS: RS: P/A :

**Laboratory Investigation:**

Hb ; Scan ;

**Serum Bilurubin** Total: Total protein :

Direct ; Albumen:

Indirect ; Globulin:

SGOT ;

SGPT ;

SAP ;

LDH ; Alpha fetoprotein:

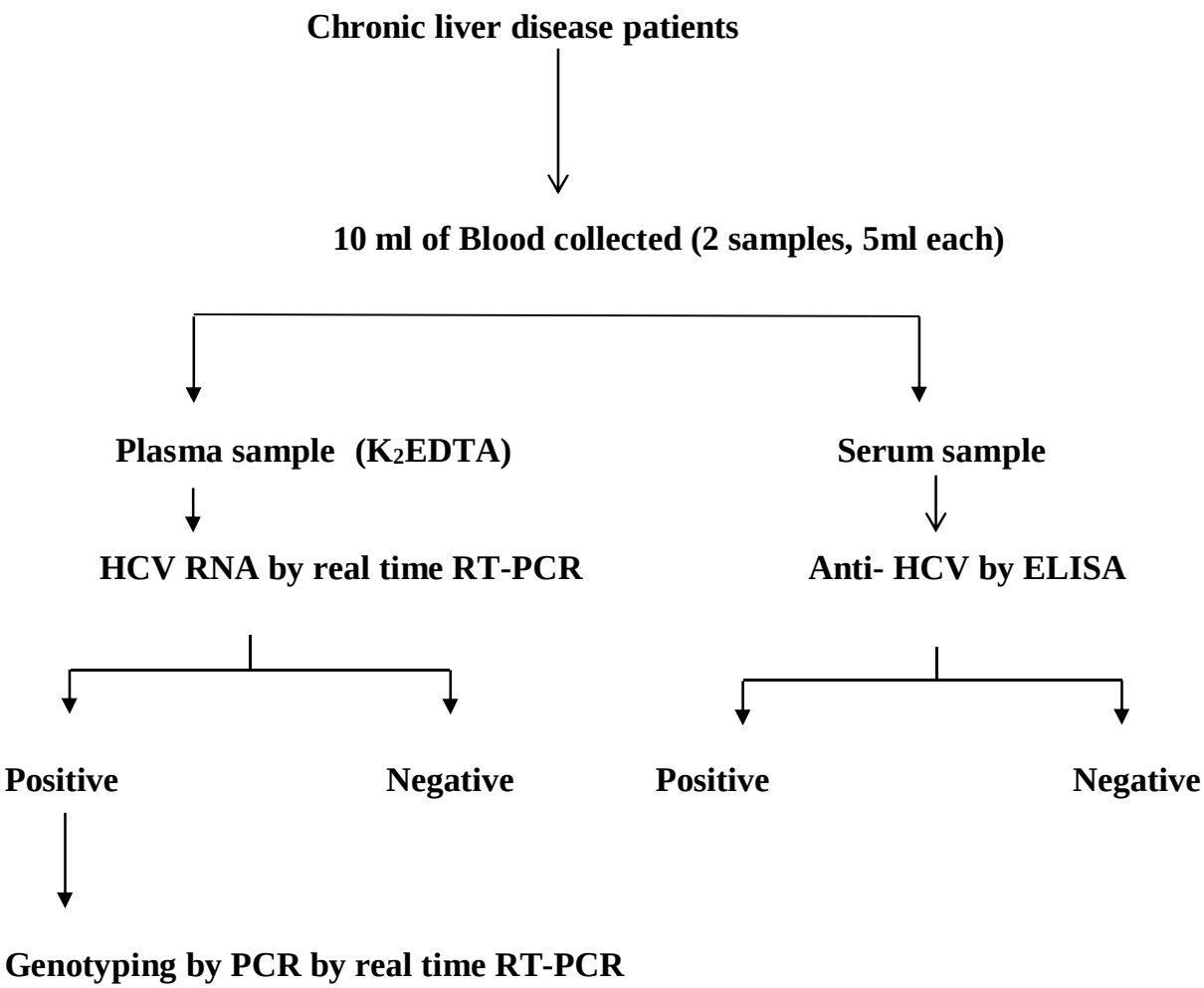
Urea ;

Creatinine ;

HCV PCR:

Genotyping:

**WORKSHEET**


Chronic liver disease patients
10 ml of Blood collected (2 samples, 5ml each)
Plasma sample (K2EDTA)
Serum sample
HCV RNA by real time RT-PCR
Anti- HCV by ELISA
Positive
Negative
Positive
Negative
Genotyping by PCR by real time RT-PCR

# *MASTER CHART*

# MASTER CHART

| S.NO | Date | Name | Age/sex | IPNO/OPNO | Diagnosis | USG | S.Bilurubin | SGPT | ALP | Anti-HCV ELISA | HCVRNA PCR | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.08.2015 | Rangasamy | 58/M | 50872/M2 | Cirrhosis | Cirrhosis | 3.8 | 58 | 105 | Negative | _ | _ |
| 2 | 1.08.2015 | Kunjukannan | 75/M | 51292/Nephro | Cirrhosis | Cirrhosis | 1.3 | 42 | 135 | Negative | _ | _ |
| 3 | 7.08.2015 | Satyamoorthy | 46/M | 52480/Nephro | Chronic hepatitis | Mild hepatomegaly | 2.1 | 102 | 225 | Negative | _ | _ |
| 4 | 7.08.2015 | Gunasundari | 54/F | 2503/MGE | Cirrhosis | Cirrhosis | 3.2 | 86 | 128 | Negative | _ | _ |
| 5 | 2.09.2015 | Muthulaksmi | 28/F | 2741/Nephro | Chronic hepatitis | Mild hepatomegaly | 5.2 | 96 | 195 | Negative | _ | _ |
| 6 | 7.08.2015 | Subammal | 45/F | 2746/M1 | Cirrhosis | Cirrhosis | 2.2 | 85 | 133 | Negative | _ | _ |
| 7 | 2.09.2015 | Muthusamy | 62/m | 57661/M2 | Cirrhosis | Cirrhosis | 1.9 | 52 | 96 | Positive | Negative | _ |
| 8 | 4.09.2015 | Rajeswari | 80/F | 72749/M2 | Cirrhosis | Cirrhosis | 1.1 | 112 | 245 | Negative | _ | _ |
| 9 | 17.09.2015 | Najjammal | 52/F | 61394/OG3 | Cirrhosis | Cirrhosis | 0.4 | 20 | 75 | Positive | Negative | |
| 10 | 18.09.2015 | Muthulaksmi | 59/F | 63239/Nephro | Cirrhosis | Cirrhosis | 1.8 | 35 | 65 | Negative | _ | _ |
| 11 | 5.10.2015 | Mahali | 65/M | 66280/Nephro | Cirrhosis | Cirrhosis | 4.2 | 122 | 235 | Negative | _ | _ |
| 12 | 7.10.2015 | Palaniammal | 62/F | 66556/M4 | Cirrhosis | Cirrhosis | 1.2 | 46 | 67 | Negative | _ | _ |
| 13 | 8.10.2015 | Nachammal | 65/F | 65136/M5 | Cirrhosis | Cirrhosis | 1.6 | 54 | 105 | Negative | _ | _ |
| 14 | 12.10.2015 | Karuppusamy | 60/M | 68103/M3 | Cirrhosis | Cirrhosis | 2.6 | 72 | 223 | Negative | _ | _ |
| 15 | 12.10.2015 | Prema | 45/F | 68566/M5 | Cirrhosis | Cirrhosis | 2.1 | 96 | 135 | Negative | _ | _ |
| 16 | 12.10.2015 | Margret | 67/F | 3280/MGE | Cirrhosis | Cirrhosis | 2.2 | 39 | 83 | Positive | Positive | 1 |
| 17 | 14.10.2025 | Chinnammal | 87/F | 68735/Ortho | Cirrhosis | Cirrhosis | 3.2 | 84 | 105 | Positive | Negative | _ |
| 18 | 14.10.2025 | Vasantha | 43/F | 67426/MG | Cirrhosis | Cirrhosis | 0.7 | 65 | 11 | Negative | Negative | _ |
| 19 | 14.10.2025 | Ayyavu.K | 42/M | 87614/MG | Chronic hepatitis | Mild hepatomegaly | 0.5 | 23 | 89 | Positive | Positive | 4 |
| 20 | 14.10.2025 | Shyam sunder | 41/M | 732/MG | Cirrhosis | Cirrhosis | 1 | 88 | 102 | Positive | Positive | 4 |
| 21 | 14.10.2025 | Senthil kumar | 39/M | 1198/MG | Cirrhosis | Cirrhosis | 2.5 | 37 | 220 | Positive | Positive | 4 |
| 22 | 14.10.2025 | Vaseela Banu | 22/F | 2461/MG | Chronic hepatitis | Mild hepatomegaly | 1.4 | 88 | 135 | Positive | Positive | 4 |
| 23 | 23.10.2015 | Balamurugan | 24/M | 501/MG | Chronic hepatitis | Mild hepatomegaly | 1.8 | 65 | 285 | Positive | Positive | 4 |
| 24 | 26.10.2015 | Kaliammal | 54/F | 7029/M6 | Cirrhosis | Cirrhosis | 0.8 | 39 | 128 | Positive | Negative | _ |
| 25 | 28.10.2015 | Sakthivel | 26/M | 53483/MGE | Chronic hepatitis | Mild hepatomegaly | 1.9 | 86 | 245 | Negative | Negative | |
| 26 | 29.10.2015 | Kumari | 66/F | 71387/nephro | Cirrhosis | Cirrhosis | 1.3 | 55 | 95 | Negative | Negative | _ |
| 27 | 30.10.2015 | Rajathi | 58/F | 3397/MG | Cirrhosis | Cirrhosis | 2.2 | 65 | 138 | Negative | Negative | _ |
| 28 | 30.10.2015 | Rajeswari | 87/F | 72749/FM1 | Cirrhosis | Cirrhosis | 8.6 | 102 | 332 | Negative | Negative | _ |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 2.11.2015 | Ranganayagi | 60/F | 3396/MGE | Cirrhosis | Cirrhosis | 2.3 | 86 | 176 | Positive | Negative | _ |
| 30 | 2.11.2015 | Subbaiyan | 73/M | 74134/Nephro | Cirrhosis | Cirrhosis | 1.9 | 42 | 167 | Negative | Negative | _ |
| 31 | 6.11.2015 | Rajesh Kumar | 27/M | 74362/M3 | Chronic hepatitis | Mild hepatomegaly | 1 | 74 | 92 | Negative | Negative | _ |
| 32 | 7.11.2015 | Solaiammal | 65/M | 74739/M2 | Cirrhosis | Cirrhosis | 1.3 | 36 | 67 | Negative | Negative | _ |
| 33 | 9.11.2015 | Palaniappan | 55/M | 77115/M3 | Cirrhosis | Cirrhosis | 2 | 54 | 175 | Positive | Negative | _ |
| 34 | 12.11.2015 | Ragupathy | 28/M | 3591/MGE | Chronic hepatitis | Mild hepatomegaly | 3.6 | 62 | 222 | Negative | Negative | _ |
| 35 | 13.11.2015 | Primathi | 55/F | MGE | Cirrhosis | Cirrhosis | 1.2 | 45 | | Positive | Positive | 1 |
| 36 | 17.11.2015 | Dhanalaksmi | 65/F | MGE | Hepatoma | Hepatoma | 2.1 | 38 | 92 | Positive | Positive | 3 |
| 37 | 17.11.2015 | Kaliathal | 65/F | 77925/M3 | Cirrhosis | Cirrhosis | 3.1 | 56 | 96 | Negative | Negative | _ |
| 38 | 17.11.2015 | Udayakumar | 48/M | 77454/M6 | Cirrhosis | Cirrhosis | 2.1 | 52 | 89 | Negative | Negative | _ |
| 39 | 18.11.2015 | Sekar | 28/M | 76979/M4 | Chronic hepatitis | Mild hepatomegaly | 6.7 | 49 | 397 | Negative | Negative | _ |
| 40 | 19.11.2015 | Lakshmi | 57/F | 77702/Med op | Cirrhosis | Cirrhosis | 2.1 | 58 | 96 | Negative | Negative | _ |
| 41 | 19.11.2015 | Thilagavathi | 46/F | 78423/MGE | Chronic hepatitis | Mild hepatomegaly | 12.3 | 102 | 332 | Negative | Negative | _ |
| 42 | 20.11.2015 | Moorthi | 35/M | 367715/MGE | Chronic hepatitis | Mild hepatomegaly | 0.6 | 64 | 102 | Positive | Negative | _ |
| 43 | 21.11.2015 | Senthilkumar | 27/M | 78970/M5 | Chronic hepatitis | Mild hepatomegaly | 0.8 | 85 | 142 | Negative | Negative | _ |
| 44 | 21.11.2015 | Papammal | 70/F | 78773/IMCU | Cirrhosis | Cirrhosis | 1.2 | 105 | 227 | Negative | Negative | _ |
| 45 | 24.11.2015 | Dhandapani | 47/M | 79014/M5 | Cirrhosis | Cirrhosis | 0.8 | 32 | 52 | Negative | Negative | _ |
| 46 | 25.11.2015 | Saraswathi | 56/F | 79185/M6 | Cirrhosis | Cirrhosis | 10.6 | 128 | 362 | Negative | Negative | _ |
| 47 | 26.11.2015 | Gnanammbal | 62/F | 74895/M2 | Cirrhosis | Cirrhosis | 0.8 | 28 | 45 | Negative | Negative | _ |
| 48 | 30.11.2015 | Ganesan | 47/M | 78493/M3 | Chronic hepatitis | Mild hepatomegaly | 0.9 | 38 | 178 | Positive | Negative | _ |
| 49 | 30.11.2015 | Sarasal | 21/F | M6 | Chronic hepatitis | Mild hepatomegaly | 2.1 | 62 | 135 | Negative | Negative | _ |
| 50 | 30.11.2015 | Kareem | 51/M | 79347/M6 | Cirrhosis | Mild hepatomegaly | 1.8 | 49 | 397 | Negative | Negative | _ |
| 51 | 1.12.2015 | Santhi | 45/F | 80240/IMCU | Cirrhosis | Cirrhosis | 2.3 | 49 | 112 | Negative | Negative | _ |
| 52 | 1.12.2015 | Ibrahim | 47/M | 81501/nephro | Cirrhosis | Cirrhosis | 4.7 | 56 | 235 | Positive | Negative | _ |
| 53 | 2.12.2015 | Paneerselvam | 57/M | 81679/M2 | Cirrhosis | Cirrhosis | 0.8 | 38 | 182 | Negative | Negative | _ |
| 54 | 2.12.2015 | Ramasamy | 75/M | 81487/M1 | Cirrhosis | Cirrhosis | 1.1 | 84 | 124 | Negative | Negative | _ |
| 55 | 4.12.2015 | Sekar | 38/M | 80291 | Chronic hepatitis | Mild hepatomegaly | 6.6 | 133 | 188 | Negative | Negative | _ |
| 56 | 4.12.2015 | Saraswathi | 65/F | 33441/MGE | Cirrhosis | Cirrhosis | 1.68 | 50 | 108 | Positive | Positive | 3 |
| 57 | 5.12.2015 | Ramayee | 75/F | 81487/M2 | Cirrhosis | Cirrhosis | 2.3 | 48 | 143 | Negative | Negative | |
| 58 | 5.12.2015 | Nagammal | 45/F | 81644/M2 | Cirrhosis | Cirrhosis | 1.2 | 66 | 175 | Negative | Negative | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 59 | 5.12.2015 | Udayakumari | 40/F | 80935/M6 | Chronic hepatitis | Mild hepatomegaly | 1.9 | 47 | 229 | Negative | Negative | |
| 60 | 11.12.2015 | Papammal | 75/F | 83659/M2 | Chronic hepatitis | Mild hepatomegaly | 3.1 | 84 | 135 | Positive | Negative | |
| 61 | 11.12.2015 | Shanmugavel | 66/M | 83413/M2 | Cirrhosis | Cirrhosis | 3.3 | 48 | 242 | Negative | Negative | |
| 62 | 12.12.2015 | Mariappan | 26/M | 84283/M5 | Chronic hepatitis | Mild hepatomegaly | 4.2 | 69 | 312 | Negative | Negative | |
| 63 | 12.12.2015 | Manian | 52/M | 84283/M5 | Cirrhosis | Cirrhosis | 1.3 | 32 | 118 | Negative | Negative | |
| 64 | 12.12.2015 | Uthiran | 45/M | 84317/M5 | Chronic hepatitis | Mild hepatomegaly | 1.9 | 45 | 363 | Positive | Positive | 3 |
| 65 | 16.12.2015 | Manickam | 57/M | 85126/S4 | Cirrhosis | Cirrhosis | 1.7 | 21 | 229 | Positive | Negative | |
| 66 | 16.12.2015 | Jeyapal | 78/M | 84524/S6 | Cirrhosis | Cirrhosis | 3.7 | 52 | 326 | Positive | Negative | |
| 67 | 17.12.2015 | Krishnammal | 53/F | 84347/M4 | Chronic hepatitis | Mild hepatomegaly | 1 | 28 | 56 | Negative | Negative | |
| 68 | 17.12.2015 | Gunasekaran | 55/M | 85666/M3 | Cirrhosis | Cirrhosis | 1.6 | 39 | 112 | Positive | Negative | |
| 69 | 17.12.2015 | Velkumar | 49/M | 2258/MGE | Cirrhosis | Cirrhosis | 2.2 | 38 | 94 | Positive | Positive | 4 |
| 70 | 18.12.2015 | Lakshmi | 33/F | 3952/MGE | Chronic hepatitis | Mild hepatomegaly | 2 | 105 | 185 | Positive | Negative | |
| 71 | 21.12.2015 | Kasi Viswanathan | 60/M | 85691/M6 | Cirrhosis | Cirrhosis | 3.6 | 82 | 332 | Positive | Negative | |
| 72 | 21.12.2015 | Danavel | 63/M | 86217/M5 | Cirrhosis | Cirrhosis | 1.2 | 34 | 63 | Negative | Negative | |
| 73 | 22.12.2015 | Arunachalam | 70/M | 86598/Nephro | Cirrhosis | Cirrhosis | 1.1 | 34 | 48 | Negative | Positive | 3 |
| 74 | 25.12.2015 | Rangasamy | 76/M | 86481/M6 | Cirrhosis | Cirrhosis | 1 | 56 | 92 | Negative | Negative | |
| 75 | 22.12.2015 | Mohan | 67/M | 94865/MGE | Hepatoma | Hepatoma | 1.2 | 24 | 401 | Positive | Positive | 3 |
| 76 | 22.12.2015 | Senthilkumar | 43/M | 82614/IMCU | Hepatitis chronic | Mild hepatomegaly | 1.9 | 39 | 122 | Positive | Negative | |
| 77 | 26.12.2015 | Radhakrishnan | 57/M | 87107/M2 | Cirrhosis | Cirrhosis | 1 | 32 | 135 | Negative | Negative | |
| 78 | 26.12.2015 | Rajamani | 63/F | 87722/M4 | Cirrhosis | Cirrhosis | 1.8 | 52 | 146 | Negative | Negative | |
| 79 | 31.12.2015 | Kunjammal | 65/F | 89105/S3 | Cirrhosis | Cirrhosis | 4.8 | 64 | 252 | Negative | Negative | |
| 80 | 31.12.2015 | Kandammal | 60/M | 88825/M2 | Chronic hepatitis | Mild hepatomegaly | 9.2 | 132 | 412 | Negative | Negative | |
| 81 | 31.12.2015 | Sivanandan | 63/M | 87816/Nephro | Cirrhosis | Cirrhosis | 1.3 | 28 | 112 | Negative | Negative | |
| 82 | 2.1.2016 | Nataraj | 65/M | 88655/M1 | Cirrhosis | Cirrhosis | 1 | 32 | 58 | Negative | Negative | |
| 83 | 4.1.2016 | Poovathal | 60/F | 39217/M4 | Cirrhosis | Cirrhosis | 8.2 | 62 | 1500 | Positive | Negative | |
| 84 | 4.1.2016 | Nishar Ahmed | 52/M | 363/MGE | Cirrhosis | Cirrhosis | 1.3 | 48 | 133 | Negative | Negative | |
| 85 | 4.1.2016 | Murugesan | 55/M | 86743/M2 | Chronic hepatitis | Mild hepatomegaly | 2.1 | 56 | 176 | Positive | Positive | 1 |
| 86 | 6.1.2016 | Narasammal | 80/F | 599/Nephro | Cirrhosis | Cirrhosis | 12.1 | 135 | 575 | Negative | Negative | |
| 87 | 6.1.2016 | Rajagopal | 38/M | 593/Nephro | Chronic hepatitis | Mild hepatomegaly | 0.6 | 82 | 158 | Positive | Negative | |
| 88 | 7.1.2015 | Gangammal | 65/F | 415/M6 | Cirrhosis | Cirrhosis | 3.2 | 62 | 162 | Negative | Negative | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 89 | 13.01.2016 | Aruna | 38/F | 2822/M2 | Chronic hepatitis | Mild hepatomegaly | 1.8 | 52 | 96 | Negative | Negative | |
| 90 | 13.01.2016 | Sekar | 45/M | 2987/M2 | Chronic hepatitis | Mild hepatomegaly | 3.8 | 92 | 256 | Negative | Negative | |
| 91 | 13.01.2016 | Arunagiri | 51/M | 85747/MGE | Cirrhosis | Cirrhosis | 2.2 | 44 | 135 | Positive | Positive | 3 |
| 92 | 18.01.2016 | Chellasamy | 66/M | 3344/Ortho | Cirrhosis | Cirrhosis | 3.6 | 52 | 148 | Negative | Negative | |
| 93 | 18.01.2016 | Subramaniam | 43/M | 2987/S3 | Chronic hepatitis | Mild hepatomegaly | 1.2 | 82 | 252 | Negative | Negative | |
| 94 | 19.01.2016 | Chinnamani | 50/F | 3371/M4 | Chronic hepatitis | Mild hepatomegaly | 3.1 | 72 | 332 | Negative | Negative | |
| 95 | 20.01.2016 | Veerammal | 65/F | 3705/M6 | Cirrhosis | Cirrhosis | 1.1 | 28 | 56 | Negative | Negative | |
| 96 | 20.01.2016 | Valiammal | 65/F | 75/MGE | Cirrhosis | Cirrhosis | 0.6 | 45 | 200 | Positive | Positive | 1 |
| 97 | 21.01.2016 | Kanniammal | 62/F | 4659/S2 | Chronic hepatitis | Mild hepatomegaly | 2.2 | 56 | 95 | Negative | Negative | |
| 98 | 23.01.2016 | Muthammal | 70/M | 4316/Nephro | Cirrhosis | Cirrhosis | 0.9 | 35 | 64 | Negative | Negative | |
| 99 | 29.01.2016 | Devaraj | 65/M | 6571/S4 | Hepatoma | Hepatoma | 2 | 45 | 214 | Positive | Positive | 3 |
| 100 | 1.2.2016 | Rajammal | 44/F | 5503/S2 | Chronic hepatitis | Mild hepatomegaly | 1.8 | 62 | 178 | Negative | Negative | |
| 101 | 2.2.2016 | Palanaal | 60/F | 7035/Nephro | Cirrhosis | Cirrhosis | 1 | 35 | 42 | Negative | Negative | |
| 102 | 2.2.2016 | Velmurugan | 53/M | 7727/M1 | Chronic hepatitis | Mild hepatomegaly | 5.2 | 82 | 345 | Negative | Negative | |
| 103 | 2.2.2016 | Mylsamy | 60/M | 7103/M2 | Cirrhosis | Cirrhosis | 1.2 | 34 | 62 | Negative | Negative | |
| 104 | 5.2.2016 | Kagarnnisha | 60/F | 7996/M2 | Chronic hepatitis | Mild hepatomegaly | 4.2 | 56 | 224 | Negative | Negative | |
| 105 | 5.2.2016 | Palaniammal | 60/F | 8683/M3 | Cirrhosis | Cirrhosis | 1 | 24 | 45 | Negative | Negative | |
| 106 | 5.2.2016 | Dharmaraj | 54/M | 8802/M6 | Cirrhosis | Cirrhosis | 3.2 | 56 | 126 | Negative | Negative | |
| 107 | 8.2.2016 | Abdul Jaffer | 67/M | 9167/M5 | Cirrhosis | Cirrhosis | 1.6 | 34 | 86 | Negative | Negative | |
| 108 | 8.2.2016 | Malliga | 68/F | 9066/IMCU | Cirrhosis | Cirrhosis | 2.2 | 56 | 236 | Negative | Negative | |
| 109 | 11.02.2016 | Pandian | 60/M | 8870/M5 | Cirrhosis | Cirrhosis | 0.6 | 28 | 45 | Negative | Negative | |
| 110 | 11.02.2016 | Rajathi | 46/F | 10013/IMCU | Chronic hepatitis | Mild hepatomegaly | 7.2 | 112 | 436 | Negative | Negative | |
| 111 | 12.02.2016 | Govindaraj | 25/M | 10152/M2 | Chronic hepatitis | Mild hepatomegaly | 2.1 | 44 | 106 | Negative | Positive | 3 |
| 112 | 12.02.2016 | Kuppammal | 47/F | 100115/M4 | Cirrhosis | Cirrhosis | 1.2 | do | 65 | Negative | Negative | |
| 113 | 15.02.2016 | Danapal | 38/M | 476/MGE | Chronic hepatitis | Mild hepatomegaly | 2.8 | 72 | 132 | Negative | Negative | |
| 114 | 15.02.2016 | Chenniammal | 65/F | 10686/M6 | Cirrhosis | Cirrhosis | 1.2 | 67 | 138 | Negative | Negative | |
| 115 | 17.2.2016 | Kumar | 44/M | 10916/MGE | Chronic hepatitis | Mild hepatomegaly | 2.8 | 72 | 295 | Negative | Negative | |
| 116 | 17.2.2016 | Gunasekaran | 56/M | 10555/M6 | Cirrhosis | Cirrhosis | 1.9 | 56 | 118 | Positive | Positive | 3 |
| 117 | 19.2.2016 | devadoss | 47/M | 11357/M3 | Chronic hepatitis | Mild hepatomegaly | 3.9 | 68 | 300 | Negative | Negative | |
| 118 | 20.02.2016 | Marimuthu | 75/M | 71808/S4 | Cirrhosis | Cirrhosis | 1.2 | 32 | 58 | Negative | Negative | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 119 | 22.2.2016 | Danalakshmi | 55/F | 10676/M6 | Chronic hepatitis | Mild hepatomegaly | 2.4 | 72 | 284 | Negative | Negative | |
| 120 | 25.2.2016 | Selvi | 47/M | 13032/M2 | Cirrhosis | Cirrhosis | 1 | 31 | 56 | Negative | Negative | |
| 121 | 01.03.2016 | Karthikeyan | 46/M | 14476/M1 | Cirrhosis | Cirrhosis | 0.8 | 36 | 52 | Negative | Negative | |
| 122 | 01.03.2016 | Muthal | 60/F | 9476/M2 | Cirrhosis | Cirrhosis | 1.3 | 40 | 65 | Negative | Negative | |
| 123 | 01.03.2016 | Solappan | 70/M | 14986/S3 | Cirrhosis | Cirrhosis | 1.2 | 35 | 48 | Negative | Negative | |
| 124 | 04.03.2016 | Rajeswari | 48/F | 15206/MGE | Chronic hepatitis | Mild hepatomegaly | 3.2 | 86 | 248 | Positive | Positive | 3 |
| 125 | 07.03.2016 | Amulraj | 54/M | 15786/M1 | Cirrhosis | Cirrhosis | 0.8 | 32 | 56 | Negative | Negative | |
| 126 | 12.03.2016 | Padravathi | 60/F | 15368/M4 | Hepatoma | Hepatoma | 2.1 | 46 | 112 | Negative | Negative | |
| 127 | 14.03.2016 | Karuppathal | 46/F | 16419/IMCU | Chronic hepatitis | Mild hepatomegaly | 6.1 | 72 | 286 | Negative | Negative | |
| 128 | 15.03.2016 | Ragunathan | 66/M | 17048/Nephro | Cirrhosis | Cirrhosis | 1.1 | 33 | 52 | Negative | Negative | |
| 129 | 16.03.2016 | Murugan | 56/M | 17645/M2 | Cirrhosis | Cirrhosis | 2.2 | 56 | 221 | Negative | Negative | |
| 130 | 16.03.2016 | Saranya | 15/F | 14204/M2 | Chronic hepatitis | Mild hepatomegaly | 0.6 | 48 | 158 | Negative | Negative | |
| 131 | 16.03.2016 | Muthu | 50/M | 406055/MGE | Chronic hepatitis | Mild hepatomegaly | 2.1 | 45 | 363 | Negative | Negative | |
| 132 | 17.03.2016 | Kavttha | 35/M | 18216/Nephro | Chronic hepatitis | Mild hepatomegaly | 8.8 | 121 | 522 | Negative | Negative | |
| 133 | 17.03.2016 | Samiammal | 62/F | 17894/Nephro | Cirrhosis | Cirrhosis | 1.2 | 36 | 62 | Negative | Positive | 1 |
| 134 | 18.03.2016 | Gopalakrishnan | 38/M | 18196/M3 | Cirrhosis | Cirrhosis | 3.2 | 56 | 185 | Negative | Negative | |
| 135 | 21.03.2016 | Karupathal | 80/F | 18757/m6 | Cirrhosis | Cirrhosis | 2.1 | 56 | 189 | Positive | Negative | |
| 136 | 21.03.2016 | Nagalakshmi | 48/F | 802/MGE | Chronic hepatitis | Mild hepatomegaly | 10.3 | 136 | 362 | Negative | Negative | |
| 137 | 21.03.2016 | Vasudevan | 35/M | 15866/MGE | Cirrhosis | Cirrhosis | 0.6 | 19 | 71 | Positive | Positive | 3 |
| 138 | 21.03.2016 | Papathy | 61/F | 18122/M3 | Hepatoma | Hepatoma | 1.3 | 80 | 102 | Positive | Negative | |
| 139 | 28.03.2016 | Sugadev | 76/M | 19950/Nephro | Cirrhosis | Cirrhosis | 1.1 | 35 | 96 | Positive | Negative | |
| 140 | 1.4.2016 | Kamala | 49/F | 20418/M6 | Chronic hepatitis | Mild hepatomegaly | 3.2 | 62 | 138 | Negative | Negative | |
| 141 | 1.4.2016 | Rajan.V | 38/M | 20071/M5 | Chronic hepatitis | Mild hepatomegaly | 2.8 | 89 | 141 | Negative | Negative | |
| 142 | 1.4.2016 | Parvathi | 70/F | 21313/M3 | Cirrhosis | Cirrhosis | 1.2 | 24 | 38 | Negative | Negative | |
| 143 | 4.4.2016 | Valliammal | 55/F | 21794/M6 | Cirrhosis | Cirrhosis | 0.8 | 32 | 54 | Negative | Negative | |
| 144 | 4.4.2016 | Manikandaprabu | 37/M | 21572/M4 | Chronic hepatitis | Mild hepatomegaly | 0.6 | 28 | 43 | Negative | Negative | |
| 145 | 5.4.2016 | Vijayalaksmi | 50/F | 22048/M1 | Chronic hepatitis | Mild hepatomegaly | 1.3 | 48 | 132 | Negative | Negative | |
| 146 | 5.4.2016 | Karuppusamy | 70/M | 21770/M6 | Cirrhosis | Cirrhosis | 1.9 | 52 | 96 | Negative | Negative | |
| 147 | 5.4.2016 | Jeya | 40/F | 22435/M2 | Cirrhosis | Cirrhosis | 4.8 | 122 | 342 | Negative | Negative | |
| 148 | 18.4.2016 | Sundararaj | 45/M | 24635/M5 | Cirrhosis | Cirrhosis | 1.1 | 35 | 68 | Negative | Negative | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 149 | 20.4.2016 | Sudha | 47/F | 25392/M2 | Chronic hepatitis | Mild hepatomegaly | 12.8 | 113 | 560 | Positive | Positive | 3 |
| 150 | 20.4.2016 | Boopathy | 60/M | 25188/M1 | Cirrhosis | Cirrhosis | 0.8 | 32 | 56 | Negative | Negative | |
| 151 | 20.4.2016 | Marudhachalam | 64/M | 24842/M2 | Hepatoma | Cirrhosis | 2.1 | 256 | 138 | Negative | Negative | |
| 152 | 21.4.2016 | Danapal | 38/M | 25570/M2 | Cirrhosis | Cirrhosis | 3.8 | 86 | 303 | Positive | Negative | |
| 153 | 28.04.2016 | Murugesan | 45/M | 26237/M6 | Cirrhosis | Cirrhosis | 0.6 | 24 | 43 | Negative | Negative | |
| 154 | 1.5.2016 | Thirunavukkarasu | 47/M | 212626/M5 | Chronic hepatitis | Mild hepatomegaly | 2.1 | 58 | 136 | Negative | Negative | |
| 155 | 3.5.2016 | Nandakumar | 60/M | 27942/M6 | Cirrhosis | Cirrhosis | 0.8 | 34 | 58 | Negative | Negative | |
| 156 | 3.5.2016 | Zenat nisha | 52/F | 28390/M2 | Cirrhosis | Cirrhosis | 1.1 | 52 | 133 | Negative | Negative | |
| 157 | 5.5.2016 | Subbulakshmi | 56/F | 28586/M2 | Cirrhosis | Cirrhosis | 1.5 | 32 | 68 | Negative | Negative | |
| 158 | 5.5.2016 | Balasundari | 52/F | 25861/M3 | Cirrhosis | Cirrhosis | 1.2 | 58 | 69 | Negative | Negative | |
| 159 | 6.5.2016 | Ganesh | 39/M | 428/MGE | Chronic hepatitis | Mild hepatomegaly | 3.2 | 76 | 102 | Positive | Positive | 3 |
| 160 | 6.5.2016 | Palani | 70/M | 28610/M2 | Cirrhosis | Cirrhosis | 2.2 | 56 | 112 | Negative | Negative | |
| 161 | 7.5.2016 | Ramasamy | 48/M | 29287/TW | Cirrhosis | Cirrhosis | 1.3 | 48 | 96 | Negative | Negative | |
| 162 | 7.5.2016 | Danabakyam | 35/F | 29190/M4 | Chronic hepatitis | Mild hepatomegaly | 3.2 | 172 | 305 | Negative | Negative | |
| 163 | 13.5.2016 | Kumaran | 60/M | 30230/Nephro | Cirrhosis | Cirrhosis | 1.8 | 50 | 82 | Negative | Negative | |
| 164 | 17.5.2016 | Muthammal | 47/F | 25655/MGE | Cirrhosis | Cirrhosis | 1.6 | 68 | 118 | Positive | Positive | 3 |
| 165 | 20.5.2016 | Palanisamy | 68/M | 31781/M3 | Cirrhosis | Cirrhosis | 2.2 | 36 | 135 | Negative | Negative | |
| 166 | 24.5.2016 | Marudhachalam | 38/M | 32521/M6 | Cirrhosis | Cirrhosis | 0.8 | 38 | 36 | Negative | Negative | |
| 167 | 27.5.2016 | Mahalalksmi | 63/F | 33518/M3 | Cirrhosis | Cirrhosis | 1.4 | 39 | 92 | Negative | Negative | |
| 168 | 28.5.2016 | Balakrishnan | 41/M | 33571/M3 | Chronic hepatitis | Mild hepatomegaly | 3.8 | 72 | 206 | Negative | Negative | |
| 169 | 2.6.2016 | Selvi | 49/F | 34417/M6 | Chronic hepatitis | Mild hepatomegaly | 2.2 | 56 | 134 | Negative | Negative | |
| 170 | 2.6.2016 | Banumathy | 44/F | 1812/MGE | Cirrhosis | Cirrhosis | 1.3 | 39 | 68 | Negative | Negative | |
| 171 | 2.6.2016 | Saraswathi | 67/F | 1798/MGE | Cirrhosis | Cirrhosis | 1.2 | 52 | 86 | Negative | Negative | |
| 172 | 3.6.2016 | Dhandapani | 52/M | 35558/M5 | Cirrhosis | Cirrhosis | 1.1 | 32 | 58 | Negative | Negative | |
| 173 | 3.6.2016 | Velammal | 63/F | MGE | Cirrhosis | Cirrhosis | 1.8 | 52 | 132 | Positive | Positive | 3 |
| 174 | 10.6.2015 | Kittammal | 70/F | 34344/M6 | Cirrhosis | Cirrhosis | 15.1 | 132 | 476 | Negative | Negative | |
| 175 | 10.6.2015 | Chinnammini | 65/M | 35403/M1 | Cirrhosis | Cirrhosis | 16 | 28 | 52 | Negative | Negative | |
| 176 | 19.6.2016 | Ramaiya | 43/M | 36030/M4 | Chronic hepatitis | Mild hepatomegaly | 3.4 | 48 | 92 | Negative | Negative | |
| 177 | 20.6.2016 | Sakthivel | 46/M | 568578/MGE | Chronic hepatitis | Mild hepatomegaly | 3.2 | 86 | 136 | Negative | Negative | |
| 178 | 20.6.2016 | Sellammal | 45/F | 35151/MGE | Cirrhosis | Cirrhosis | 1.6 | 48 | 106 | Negative | Negative | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 179 | 22.6.2016 | Sathiskumar | 43/M | 39360/IMCU | Chronic hepatitis | Mild hepatomegaly | 3.1 | 64 | 208 | Positive | Negative | |
| 180 | 30.6.2016 | Nataraj | 62/M | 40350/S4 | Cirrhosis | Cirrhosis | 2.1 | 56 | 84 | Positive | Positive | other than 1,2,3,4 |
| 181 | 30.6.2016 | Indrani | 62/F | 40793/M1 | Chronic hepatitis | Mild hepatomegaly | 3.8 | 96 | 192 | Positive | Negative | |
| 182 | 6.7.2016 | Muthammal | 75/F | 39125/M5 | Cirrhosis | Cirrhosis | 3.1 | 62 | 118 | Negative | Negative | |
| 183 | 11.7.2016 | Rangaraj | 66/M | 40158/M3 | Cirrhosis | Cirrhosis | 1 | 32 | 45 | Negative | Negative | |
| 184 | 11.7.2016 | Nalani | 55/F | 42136/Nephro | Cirrhosis | Cirrhosis | 1.3 | 52 | 86 | Positive | Positive | other than 1,2,3,4 |
| 185 | 13.7.2016 | Bagyalaksmi | 50/F | 44158/M1 | Chronic hepatitis | Mild hepatomegaly | 3.2 | 86 | 201 | Positive | | |
| 186 | 25.7.2016 | Ganesan | 51/M | 47650/M5 | Cirrhosis | Cirrhosis | 1.2 | 38 | 62 | Positive | Positive | 3 |
| 187 | 25.7.2016 | Lakshmi | 63/F | 43361/MGE | Cirrhosis | Cirrhosis | 2.3 | 72 | 153 | Positive | | |
| 188 | 28.7.2016 | Narasimma Rhagavan | 51/M | 33521/MgE | Hepatoma | Hepatoma | 4.1 | 112 | 158 | Positive | Positive | 3 |
| 189 | 28.7.2016 | Abdul Ajeesh | 72/M | 33578/Mge | Cirrhosis | Cirrhosis | 3.2 | 56 | 98 | Positive | Positive | 3 |
| 190 | 28.7.2016 | Annapushpam | 70/M | 33081/MGE | Cirrhosis | Cirrhosis | 5.6 | 48 | 351 | Positive | Positive | 3 |
| 191 | 30.7.2016 | Nagarajan | 50/M | 33543/MGE | Cirrhosis | Cirrhosis | 1.5 | 64 | 145 | Positive | Positive | 3 |
| 192 | 1.8.2016 | Sarojini | 55/F | 19119/M2 | Cirrhosis | Cirrhosis | 1.2 | 32 | 65 | Negative | | |
| 193 | 1.8.2061 | Thangavel | 53/M | 18105/Nephro | Cirrhosis | Cirrhosis | 3.6 | 59 | 583 | Negative | | |
| 194 | 2.8.2016 | Rangammal | 48/F | 34161/M2 | Chronic hepatitis | Mild hepatomegaly | 1.3 | 46 | 89 | Negative | | |
| 195 | 5.8.2016 | Mariammal | 56/M | 34126/M5 | Cirrhosis | Cirrhosis | 0.9 | 34 | 68 | Negative | | |
| 196 | 5.8.2016 | Andappan | 68/M | 336912/M1 | Cirrhosis | Cirrhosis | 3.6 | 58 | 117 | Negative | | |
| 197 | 5.8.2016 | Raji | 52/F | 34587/M4 | Cirrhosis | Cirrhosis | 9.2 | 125 | 465 | Negative | | |
| 198 | 9.8.2016 | Dharmalingam | 47/M | 34298/M4 | Cirrhosis | Cirrhosis | 1.7 | 32 | 108 | Negative | | |
| 199 | 9.8.2016 | chellamal | 48/M | 33465/M3 | Chronic hepatitis | Mild hepatomegaly | 4.3 | 84 | 226 | Negative | | |
| 200 | 10.8.2016 | Sabariammal | 63/F+D75 | 35214/M6 | Cirrhosis | Cirrhosis | 1.6 | 56 | 228 | Negative | | |